Hypertension Induces Heightened Activity of the Adaptive Immune System

Tuantuan Zhao, Dept of Biomedical Sciences. Cedars-Sinai Medical Ctr, Los Angeles, CA; Xiao Z Shen, Dept of Physiology, Zhejiang Univ Sch of Med, Hangzhou, Zhejiang, China; Ellen A Bernstein, Kenneth E Bernstein, Dept of Biomedical Sciences. Cedars-Sinai Medical Ctr, Los Angeles, CA

Adaptive immunity plays a key role in the pathogenesis of hypertension, but how does hypertension affect immunity? To study this, splenic dendritic cells (DC) and peritoneal macrophages were isolated from normotensive and hypertensive angiotensin II (AngII)-infused C57BL6/J mice (490 ng/kg/min, 2 wk). These antigen presenting cells (APCs) were loaded with ovalbumin (OVA) or the OVA MHC class I epitope SIINFEKL (SKL) for 3h. Then, splenocytes from OT-I mice, containing OVA-specific, CD8+ T cells (OT-I cells), were added for 4h. Flow cytometry showed significantly more activated T cells expressing CD69 when stimulated with APCs from AngII-treated mice than equivalent cells from naïve mice (As example, DC+OVA: 2.9 ± 0.4% vs. 1.8 ± 0.3%, P<0.05; DC+SKL: 13.9 ± 0.9% vs. 7.4 ± 1.1%, P<0.01). To study antigen presentation in vivo, we immunized AngII and sham treated mice with OVA and adjuvant. Consistently more OVA-specific CD8+ T cells were induced in the blood (2.7 ± 0.2% vs. 1.2 ± 0.4%, P<0.05) and the spleen (2.6 ± 0.3% vs. 1.5 ± 0.2%, P<0.05) of hypertensive mice vs. sham when measured by flow using a H-2kβ-SKL tetramer. RIP-mOVA mice were also used to study if hypertension affects APC cross-presentation of self-antigens. This transgenic mouse line expresses membrane-bound OVA in pancreatic islet β cells and kidney proximal tubular cells. When OT-I cells (5x10⁶) are injected into RIP-mOVA mice, they activate by cross-presentation of OVA and cause insulitis and diabetes. RIP-mOVA mice were made hypertensive with either AngII or L-NAME. When OT-I cells were infused into hypertensive RIP-mOVA mice, they rapidly developed much higher blood glucose levels as compared to equivalently treated normotensive RIP-mOVA mice. For example, comparing mice 3 weeks after AngII and 1 wk after OT-I cells to normotensive mice 1 wk after OT-I cells, blood glucose was 331 ± 47 mg/dl in the hypertensive mice vs. 168 ± 39 mg/dl in the normotensive controls. Also, far more pancreatic islets were infiltrated by T cells in the hypertensive mice (AngII 78%, 31 of 40; L-NAME 72%, 21 of 29; control 13%, 10 of 75). In conclusion, hypertension itself is associated with higher activity of APC presentation of foreign and self-antigens which may explain why hypertension induces inflammation.

T. Zhao: None. X.Z. Shen: None. E.A. Bernstein: None. K.E. Bernstein: None.

Funding: No

Funding Component:

Adoptive Transfer of CD8 T Cells With Constitutively Active Pi3ky Induces Hypertension in Mice

Daniela Carnevale, Sapienza Univ of Rome at IRCCS Neuromed, Pozzilli (IS), Italy; Maria Piacenti, Giuseppe Cifelli, Roberta Iacobucci, IRCCS Neuromed, Pozzilli (IS), Italy; Giuseppe
In the field of research exploring the connection existing between hypertension and immune system, CD8 effector T cells emerge as the possible mediators of target organ colonization. In the absence of overt inflammation or pathogen response, naïve T cells circulate from the blood into secondary lymphoid organs, where, upon challenge, become activated. Then, they differentiate into effector T cells, which display typical activation patterns. However, less is known about the intracellular signaling pathways that are responsible for the acquisition of effector functions in T cells. The p110γ isoform of the PI3K family has unique features, being crucially involved in the immune and cardiovascular systems. On this issue, we have described that PI3Kγ has a crucial role in blood pressure regulation, being KO mice protected from AngII-induced hypertension. Moreover, we found that mice with a constitutively active PI3Kγ isoform (CAAX mice) were spontaneously hypertensive (SBP: CAAX 135 ± 3 vs WT 105 ± 4 mmHg, p<0.001). Interestingly, PI3Kγ is known to play a selective role in regulating the migration of effector CD8+ T cells, even though there was no effect of PI3Kγ in naïve T cells. Thus we explored the possible involvement of PI3Kγ in the crosstalk between hypertension and immunity. CAAX mice displayed a significant infiltration of activated CD8⁺CD69⁺ T cells in kidney, as compared to WT mice (10.2 ± 2.1 vs 2.8 ± 0.6 *10⁴ cells/kidney, p<0.01). At the functional level, this phenotype was associated with enlarged Bowman’s spaces and fibrosis in the kidney of CAAX mice, leading to disruption of renal function, as shown by later development of proteinuria. In the end, to demonstrate whether the CAAX hypertensive phenotype, associated to renal damage after CD8 colonization, could be ascribed to the overactivation of PI3Kγ signaling in this immune cell type, we performed an adoptive transfer of CD8 T cells isolated from CAAX mice in WT mice. Strikingly we found that CD8 T cells with constitutively active PI3Kγ were effective to induce hypertension in naïve mice. These data suggest that in the development of hypertension, PI3Kγ signaling in CD8 T cells is crucial for their accumulation in the kidney, likely contributing to increase in blood pressure by altering renal function.


Funding: No

Funding Component:

003

Direct and Indirect Effects of Prostaglandin E₂ and Its EP1/EP3 Receptors on Dendritic Cell Activation in Mice with L-NAME/High Salt-induced Hypertension

Liang Xiao, Hana A Itani, Maria P Kraemer, Richard M Breyer, David G Harrison, Vanderbilt Univ, Nashville, TN

We recently identified a pathway underlying immune activation in hypertension. Proteins oxidatively modified by reactive γ-ketoaldehydes (isoketals) accumulate in dendritic cells (DCs). These are immunogenic and lead to subsequent T lymphocytes activation. The local signals that stimulate DCs to accumulate isoketal adducts remain undefined. Prostaglandin E₂ (PGE₂) has been implicated in the inflammation associated with hypertension. We hypothesized that PGE₂ via its EP3 receptor contributes to DC activation in hypertension. EP3⁻/⁻ mice and wild type (WT) littermates were exposed to sequential hypertensive stimuli involving an initial 2-week
exposure to the NOS inhibitor L-NAME (LN) in drinking water, a 2 week washout period, and a subsequent 4% high salt diet (HS) for 3 weeks. In WT mice, this protocol increased systolic pressure from 123±2 to 148±8 mmHg (p<0.05), and renal CD4+ and CD8+ effector memory T cells by 2 to 3 fold. This was associated with a striking accumulation of isoketal protein adducts in splenic DCs. However, the increases in blood pressure, renal T cell infiltration and DC isoketal formation were completely prevented in EP3−/− mice. We further hypothesized that EP3 receptors contribute to oxidative stress production in the kidney. As measured by dihydroethidium with confocal microscopy, the LNHS protocol induced marked increases in superoxide production in WT mice, but not in EP3−/− mice. To examine the direct effects of PGE2, splenic DCs were incubated with PGE2 in vitro for 24 hours. PGE2 dose-dependently increased isoketal-adduct formation in DCs (vehicle: 8.8±5.1% vs. 50 nM PGE2: 41.4±11.7%, p<0.05). Interestingly, this effect was not blocked by the EP3 receptor antagonist DG-041 (30 nM), but was completely prevented by the EP1 receptor blocker SC-51322 (20 μM). These data indicate both direct and indirect roles of PGE2 in DC activation in hypertension. In vivo, PGE2 has a predominant effect on EP3 receptors to enhance renal vascular ROS production, which likely leads to isoketal-adduct formation and accumulation in DCs. PGE2 also acts directly on DCs via its EP1 receptors to stimulate intracellular isoketal formation. Together, these findings provide additional information as to how PGE2 modulates inflammation in hypertension.

Isha S Dhande, Mykola Mamenko, Yaming Zhu, Oleh Pochynyuk, Univ of Texas Health Science Ctr, Houston, TX; Scott Wenderfer, Michael Braun, Baylor Coll of Med, Houston, TX; Peter Doris, Univ of Texas Health Science Ctr, Houston, TX

The genetic mechanism of end organ injury in hypertension may involve gene variation in genes participating in inflammation and its regulation. We identified a novel truncating mutation in the hypertensive end organ injury-prone spontaneously hypertensive rat (SHR-A3/SHRSP) line affecting the C-terminus of STIM1, a protein involved in the store-operated Ca2+ entry (SOCE) pathway. The genomes of injury-resistant SHR lines, including SHR-B2 used here, encode the ‘wild-type’ STIM1. SOCE is required by T cells to activate the transcription factor NFAT and regulate T cell proliferation and cytokine production. T cell receptor (TCR) stimulation depletes intracellular Ca2+ stores, activates the ER Ca2+-sensor STIM1 and results in SOCE. We tested the effect of STIM1 mutation on lymphocyte SOCE and found it was dramatically reduced in SHR-A3, but not in SHR-B2 (maximal [Ca2+]i: 150.5±34.9 vs 521.5±42.6 nM, p<0.0001). Flow cytometric analysis of circulating T cell subsets revealed comparable levels of CD4+ and CD8+ T cells in both lines, however circulating CD4+CD25+FoxP3+ Tregs were reduced in SHR-A3 compared to SHR-B2 (4.34±0.59 vs 7.12±0.33%, p=0.01). TCR-induced lymphocyte proliferation was similar in both SHR-A3 and SHR-B2. T cell cytokine production in response to TCR stimulation was markedly impaired CD4+ T cells from SHR-A3 compared with SHR-B2 (IL-2: 168±83.4 vs 1385±377.0 pg/mL, p=0.01; IFNγ: 235±69.5 vs 2119±434.7 pg/mL, p=0.002). IL-2 and IFNγ production was completely inhibited.

Funding: Yes
Funding Component: National Center
004
by Pyr6, an inhibitor of Stim1-dependent SOCE. However, circulating levels of IL-2 and IFNγ were not different between the two lines. Based on our findings, we conclude that TCR-mediated effector signaling is impaired due to defective SOCE in SHR-A3 rats. Defects in SOCE in SHR-A3 attributable to STIM1 mutation alter T cell function, reduce T_reg numbers and may disturb regulatory interactions between T cells and other immune cells involved in end organ injury.


Funding: No

Funding Component:

005

A Small-molecule Inhibitor of NLRP3 Inflammasome Activity, MCC950, Reduces Blood Pressure and Restores Renal Function in Hypertensive Mice

Dorota M Ferens, Shalini M Krishnan, Michelle M Kett, Yeong H Ling, Katrina M Mirabito, Monash Univ, Clayton, Australia; Avril A Robertson, Matthew A Cooper, The Univ of Queensland, Brisbane, Australia; Antony Vinh, Christopher T Chan, Monash Univ, Clayton, Australia; Ashley Mansell, Hudson Inst of Medical Res, Clayton, Australia; Christopher G Sobey, Grant R Drummond, Monash Univ, Clayton, Australia

Inflammasomes are a family of interleukin-1 processing complexes and master regulators of inflammation. We recently showed that both one-kidney/deoxycorticosterone acetate/salt (1K/DOCA/salt)- and angiotensin II-dependent hypertension in mice are associated with elevated expression of the NLRP3 inflammasome in the kidneys. Moreover, genetic deficiency of a key subunit critical for NLRP3 inflammasome activity protected mice against renal inflammation and chronic pressor responses associated with these models. As a step towards translation of these findings into new therapies, here we investigated whether a highly specific small-molecule inhibitor of NLRP3 inflammasome activity, MCC950, similarly reduces the deleterious effects of 1K/DOCA/salt on blood pressure (BP) and renal function. Male C57BL6/J mice were implanted with telemetry probes for continuous recording of BP (mean arterial (MAP), systolic, diastolic) and heart rate (HR), or placed in metabolic cages for 24 h urine collections to assess renal function. Once baseline parameters were established, mice were uninephrectomized, received a DOCA pellet (2.4 mg/kg/d, s.c.) and were given 0.9% saline to drink. Following establishment of hypertension (10 d), mice were implanted with osmotic pumps containing either MCC950 (10 mg/kg/d, s.c.) or vehicle (saline) and followed for 28 d. MAP increased from 102 ± 2 to 133 ± 3 mmHg over the 10 d following 1K/DOCA/salt surgery. In vehicle-treated mice, MAP remained at this elevated level until the end of the treatment period. By contrast, MAP of mice treated with MCC950 gradually declined such that at day 38 it was 15 mmHg lower than that of vehicle-treated mice. Systolic and diastolic BP response was similar to MAP, whereas HR was unaffected by MCC950. Urine, Na+ and albumin excretion, and osmolality were all markedly increased after 10 d of 1K/DOCA/salt treatment. Consistent with its effect on BP, MCC950 decreased each of these parameters by 30-40%, whereas vehicle had no effects. In conclusion, we have shown that an inhibitor of NLRP3 inflammasome activation reduces BP and restores renal function in mice with established hypertension, highlighting MCC950 as a promising candidate for future therapies.
Let-7b Mirna Down-regulates Cytochrome P450 Epoxygenase Ii High Fat Diet-induced Obesity

Ahmed A Elmarakby, Mohamed A Katary, Ahmed S. Ibrahim, Babak Baban, Mohamed Al-Shabrawey, Augusta Univ, Augusta, GA

Obesity-induced vascular inflammation is an early pathological change for the development of diabetic nephropathy. Studies have previously demonstrated that high fat diet (HFD) treatment decreased cytochrome P450 epoxygenase-mediated epoxyeicosatrienoic acids (EETs) production which in turn triggers vascular dysfunction and renal inflammation. We hypothesize that HFD up-regulates let-7b miRNA to decrease epoxygenase-mediated EETs production triggering vascular dysfunction and renal injury. Injection of let-7b mimetic has let-7b (1 nmol/day i.v) into rats decreased renal and hepatic cyp2c23 and cyp2j epoxygenases expression (P< 0.05). To determine whether high fat diet up-regulates let-7b to decrease EETs production, we use sEH gene (Ephx2) knock-out mice (KO) as a model for high EETs availability as EETs are rapidly hydrolyzed by the soluble epoxide hydrolase (sEH) to less active metabolites. WT and Ephx2 (-/-) mice were fed normal (ND, 14% fat) or HFD (60 % fat) for 3 months. HFD treatment was associated with 2 fold up-regulation of renal let-7b miRNA and this effect was coincided with down-regulation of cyp2c44 and cyp2j epoxygenases and decreased EETs levels. Decreased EETs levels in HFD fed rats was also associated with significant podocyte loss and elevation in podocalyxin excretion (56±4 ng/day) when compared to rats fed ND (33±7 ng/day) and these changes were significantly reduced in Ephx2 (-/-) mice. Furthermore, HFD treatment markedly elevated T helper cells expressing inflammatory IL-17 (CD3+CD4+TH17+) and decreased anti-inflammatory regulatory T cells (Tregs, CD3+CD4+FOXP3+) when compared to ND and these changes were also significantly attenuated in Ephx2 (-/-) mice. In-vitro, treatment of cultured glomerular endothelial cells with palmitate (200 μM) for 48 hours increased let-7b miRNA expression and decreased cyp2j mRNA and the tight junction protein ZO-1 expression levels and these changes were significantly reduced by transfecting cells with let-7b inhibitor (50 nM). Our data suggest that up-regulation of let-7b miRNA decreases epoxygenase-mediated EETs production to trigger vascular dysfunction and inflammation in the kidney of obese mice via shifting T cells polarization to favor inflammatory Th17 cells activation.

TRPM7-kinase Modulates Renal and Splenic Macrophage and T-lymphocyte Infiltration in Aldosterone-salt-induced Hypertension

Francisco J Rios, Katie Y Hood, Adam Harvey, Univ of Glasgow, Glasgow, United Kingdom; Karla B Neves, Univ of Sao Paulo, Ribeirao Preto, Brazil; Panagiota Anyfanti, Aristotle Univ of Thessaloniki, Thessaloniki, Greece; Ryszard
TRPM7 is a Mg\(^{2+}\) channel linked to a kinase domain important in cell proliferation and survival. We demonstrated that cells deficient in TRPM7-kinase domain are prone to aldosterone (aldo)-induced oxidative stress and inflammation. Here, we investigated whether TRPM7-kinase plays a role in inflammatory responses in aldo-induced hypertension. Wild-type (WT) or heterozygote TRPM7-kinase domain (TRPM7+/−) mice were infused with aldo (600µg/Kg/day - osmotic minipumps) and 1% NaCl in the drinking water (aldo-salt) for 4 weeks. Inflammatory responses were evaluated by examining T cells (CD4+ and CD8+) and macrophages (M1- and M2-phenotype) infiltration in kidneys and spleens, using flow cytometry. Gene and protein expression was assessed by real-time PCR and immunoblot respectively. ROS was evaluated by lucigenin chemiluminescence. Aldo-salt increased kidney mass and urinary levels of albumin, Ca\(^{2+}\), Mg\(^{2+}\) and K\(^{+}\), similarly in WT and TRPM7+/− (p<0.05 vs controls). Kidneys from TRPM7+/− mice presented a higher total number of inflammatory infiltrated cells (0.87x10\(^6\) vs WT 0.22x10\(^6\) cells/g), TCD4+ cells (27% vs WT 19%), and macrophages (47% vs WT 31%) (p<0.05 vs controls). Kidneys from WT aldo-salt showed increased ROS production (1.7-fold) and Nox2 (2-fold) protein expression (p<0.05 vs control) and presented similar cell infiltration to TRPM7+/− mice. Kidneys from TRPM7+/− aldo-salt showed increased M2-macrophages CD206+ (4.3% vs WT 2.3%) and mRNA expression for the anti-inflammatory cytokine IL-10 (55% vs WT), whereas decreased the expression of the pro-inflammatory TNF\(\alpha\) (30% vs WT) and Nox2 protein (1.8-fold). Spleen mass was increased by 40% only in WT aldo-salt. Spleens from untreated TRPM7+/− showed increased infiltrated inflammatory cells (3.7x10\(^5\) vs WT 2.0x10\(^5\) cells/mg). Splenic macrophages were higher in untreated TRPM7+/− (8.8% vs WT 5.9%) and presented an increase in CD206 M2-marker (16% vs WT 7%), higher TCD4+ (68% vs WT 50%) and TCD8+ (26% vs WT 17%), values that were similar to WT aldo-salt. Our data provide insights into the importance of the TRPM7-kinase domain in the immune system activation, which when down regulated provokes an increase in inflammatory cell infiltration in kidneys and spleens in aldo-salt-induced hypertension.

Depletion of Antibody-Secreting Plasma Cells Attenuates Hypertension in an Experimental Model of Autoimmune Disease

Erin B. Taylor, Michael J. Ryan, Univ of Mississippi Medical Ctr, Jackson, MS

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that is characterized by aberrant immunoglobulin (Ig) production, notably pathogenic autoantibodies, and is associated with prevalent hypertension, renal injury, and cardiovascular disease. Recent studies by our laboratory have shown that chronic B cell depletion with anti-CD20 monoclonal antibody reduces autoantibody production and prevents the development of hypertension in an experimental female mouse model of SLE (NZBWF1). However, the treatment was only effective when administered before the onset of autoantibody
production. Because long-lived plasma cells produce the majority of serum Ig and are the primary source of autoantibodies in SLE, we hypothesized that depletion of plasma cells using the proteasome inhibitor bortezomib would lower autoantibody production and attenuate hypertension. Thirty week old female NZBWF1 and control (NZW) mice were injected i.v. with vehicle (0.9% saline) or bortezomib (0.75 mg/kg) twice weekly for four weeks. Percentages of CD138+ intracellular-κ light chain+ plasma cells in the bone marrow were lower in bortezomib treated SLE mice compared to vehicle-treated SLE mice (1.7±0.19% vs. 0.95±0.18%, p<0.05), as assessed by flow cytometry. Total plasma IgG was higher in SLE mice as compared to control mice (5.02±1.2 mg/mL vs. 2.88±0.78, p<0.05), and were lower in SLE mice treated with bortezomib (1.5±0.5 mg/mL, p<0.05 vs. SLE-vehicle). In addition, bortezomib treatment reduced circulating anti-dsDNA IgG levels in SLE mice (OD450 1.36±0.25 vs. 0.44±0.11  p<0.01). Urinary albumin excretion, an indicator of glomerular injury, was increased in SLE mice as compared to control mice (16.8±10.1 vs. 0.015±0.002 mg/day, p<0.05) and was lower in SLE mice treated with bortezomib (0.24±0.016 mg/day, p<0.05 vs. SLE-vehicle). Mean arterial pressure (MAP; mmHg) measured in conscious mice by carotid artery catheter was higher in SLE mice than in control mice (142±5 vs. 118±3, p<0.001). MAP was significantly lower in SLE mice treated with bortezomib when compared to vehicle treated mice (119±4 vs. 142±5, p<0.001). These data suggest that production of autoantibodies by plasma cells in SLE mechanistically contribute to the pathogenesis of hypertension.

E.B. Taylor: None. M.J. Ryan: None.

Funding: No

Funding Component: 009

Genetically Modified Probiotics for Oral Delivery of Angiotensin-(1-7) Confers Protection Against Pulmonary Hypertension

Colleen Cole Jeffrey, Vinayak Shenoy, Amrisha Verma, Victor Aquino, Ashok Kumar, Zhibing Liang, Qiuhong Li, Michael Katovich, Mohan Raizada, Univ of Florida, Gainesville, FL

Background: Previous studies have established that activation of the members of the vasoprotective axis of the renin-angiotensin system [Angiotensin Converting Enzyme 2 (ACE2) or Angiotensin-(1-7) (Ang-(1-7))] prevents and arrests progression of pulmonary hypertension (PH) pathophysiology. Our objective in this present study was to generate a probiotic containing Ang-(1-7) and test the hypothesis that oral administration of such a probiotic would provide cardiopulmonary protection against PH. Methods: In this study, we genetically modified the commensal bacterium Lactobacillus paracasei (LP), commonly found in the gut and used as a probiotic, to serve as a live vector for the oral delivery of Ang-(1-7) and investigated its therapeutic potential in attenuating PH. The vectors pTRKH3-ldh-SP-GFP or pTRKH3-ldh-SP-Ang-(1-7) were introduced by electroporation into LP. PH was induced by a single injection of monocrotaline (MCT; 50 mg/Kg s.c) in rats. A subset of animals was orally gavaged every other day for four weeks with 1x10^9 CFU of LP, LP secreting GFP (LP-GFP), or LP secreting Ang-(1-7) (LP-A). Results: Oral feeding of LP-A significantly reduced MCT-induced right ventricular systolic pressure (RVSP) by 43% (Control: 27±1; MCT: 76±8; MCT+LP: 56±6; MCT+LP-GFP: 59±7; MCT+LP-A: 43±3 mmHg) and RV hypertrophy by 33% (Control: 0.25±0.01; MCT: 0.6±0.02; MCT+LP: 0.48±0.04; MCT+LP-GFP: 0.48±0.04; MCT+LP-A: 0.41±0.03). Moreover, LP-A feeding restored cardiac contractility (Control: 2070±95; MCT:...
313±295; LP-A: 2060±119 mmHg/s) and attenuated myocardial fibrosis. These beneficial effects on the cardiopulmonary system were associated with profound changes in gut pathology. MCT-induced PH was associated with an increase in ileum villus length and thickening of proximal colon, and a decrease in goblet cells/villus area, all of which indicate intestinal injury and altered immune status. However, these parameters were significantly attenuated by oral feeding of LP-A. Conclusions: Oral administration of a genetically modified commensal bacterium that can secrete Ang-(1-7) provides cardiopulmonary protection against PH. Thus, delivery of Ang-(1-7) by probiotic means could be considered an innovative therapeutic strategy for PH.


Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

CD44 and CD44+ Cells are Dispensable for the Recruitment of Renin Expressing Cells

Maria Luisa S Sequeira Lopez, Brian C Belyea, Rajwinderjit Kaur, Silvia Medrano, R. Ariel Gomez, Univ of Virginia, Charlottesville, VA

In response to a homeostatic stress the number of cells that make renin increases dramatically along the renal arteriolar tree resembling the embryonic pattern. We have shown that this “recruitment” occurs by re-expression of renin in smooth muscle cells that differentiated from embryonic renin cells. A recent study proposed that during recruitment, renal CD44+ mesenchymal stem-like cells can differentiate into juxtaglomerular (JG)-like renin-producing cells. To test such hypothesis, we assessed the distribution and role of CD44+ cells in renin cell recruitment. Mice with homozygous (KO) and heterozygous (het) deletion of CD44 (knockin for LacZ) were treated with low-sodium diet (0.05%) plus captopril (0.5 g/l) for 10 days (n: 9 treated, 7 controls). Body and kidney weights and BP were not different between KO and het mice. BUN and creatinine were significantly increased in both KO and Het treated mice. The number of renin expressing cells in the kidney and circulating renin increased similarly in treated mice (ELISA, untreated: het 131,503 ± 19,319 pg/mL vs KO 84,714 ± 29,065 pg/mL p=0.2517; treated: het 367,850 ± 38,189 pg/mL vs KO 495,120 ± 80,311 pg/mL p=0.2311). Interestingly, immunostaining for CD44 was negative in kidneys of untreated and treated wild type mice. We occasionally observed in CD44-LacZ het or KO mice isolated LacZ positive cells inside the glomeruli (1 or less per sagittal kidney section) and none in the JG area. On the other hand, immunostaining for CD44 on kidney sections of Ren1cKO mice revealed positive cells within perivascular infiltrates. To confirm these results we performed qRT-PCR for CD44 on kidney samples from CD44 het and KO treated, untreated, control, and Ren1cKO mice. CD44 mRNA expression confirmed the histological findings. In summary: 1) CD44 is dispensable for renin expression and recruitment, and 2) CD44+ cells do not contribute to the pool of renin expressing cells in the kidney during basal conditions or in response to a homeostatic stress as previously suggested. However, they do participate in the inflammatory process observed surrounding the vessels in mice with deletion of the renin gene, suggesting that they derived from the circulation and not from the kidney.

M.S. Sequeira Lopez: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants
An Orally Absorbable Potent NHE3 Inhibitor Attenuates Angiotensin II-induced Hypertension Primarily by Inhibiting NHE3 in the Proximal Tubule of the Kidney

Xiao C Li, Hoang Nguyen, Jia L Zhuo, Univ of Mississippi Medical Ctr, Jackson, MS

We have recently shown that angiotensin (ANG II)-induced hypertension was attenuated in mice with global (Nhe3/−) and Nhe3/− mice with transgenic rescue of the NHE3 gene selectively in small intestines (tgNhe3/−), suggesting an important role of NHE3 in the development of ANG II-dependent hypertension. In this study, we specifically tested whether the pharmacological inhibition of NHE3 mainly in the proximal tubules of the kidney attenuates ANG II-dependent hypertension induced by a low and slow pressor dose of ANG II supplemented with a high salt diet. Overall, 9 groups (n=5-12) of adult male C57BL/6J mice were infused with or without ANG II (500 μg/kg/day, i.p. via minipump) and supplemented with or without a 2% NaCl diet to slowly and moderately increase systolic blood pressure (SBP) in 2 weeks. ANG II alone increased SBP from 116 ± 2 mmHg to 140 ± 2 mmHg (p<0.01), and supplement of ANG II with a 2% NaCl diet further increased SBP to 147 ± 4 mmHg (p<0.05). Concurrent treatment with an orally active, absorbable NHE3 inhibitor AVE0657 (Sanofi-Aventis; 20 mg/kg/day, p.o.) significantly decreased SBP to 125 ± 4 mmHg in ANG II-infused mice (p<0.01), and to 134 ± 6 mmHg in ANG II-infused mice supplemented with 2% NaCl (p<0.01), respectively. Further treatment with AVE0657 and losartan, an AT1 receptor blocker (20 mg/kg/day, p.o.), completely normalize SBP in mice treated with ANG II and 2% NaCl to control (115 ± 5 mmHg, p<0.01). In the kidney, AVE0657 significantly increased 24h urinary Na+ excretion from 157.1 ± 6.7 to 207.7 ± 8.1 μmol/24h (p<0.01) without altering 24h urine excretion or SBP. Furthermore, AVE0657 did not significantly alter 24 h fecal Na+ excretion in non ANG II-infused (4.99 ± 0.37 μmol/24h, n.s.) or ANG II-infused mice (4.19 ± 0.67 μmol/24h, n.s.), compared with control (4.02 ± 0.20 μmol/24h, n.s.) or global Nhe3/− mice (50.8 ± 0.8 μmol/24h, p<0.01). Since small intestines in the gut and the proximal tubules of the kidney express the vast majority of NHE3 in the body, these results provide preclinical evidence and perspectives that orally absorbable NHE3 inhibitors may be pharmacologically beneficial to prevent and treat hypertension induced by ANG II and a high salt, mainly by inhibiting NHE3 in the proximal tubule of the kidney.

X.C. Li: None. H. Nguyen: None. J.L. Zhuo: None.

Funding: No

Funding Component:

011

Involvement of Drp1, A Mitochondrial Fission Inducer, in Angiotensin II-induced Hypertensive Vascular Remodeling in vitro and in vivo

Xiao C Li, Hoang Nguyen, Jia L Zhuo, Univ of Mississippi Medical Ctr, Jackson, MS

We have recently shown that angiotensin (ANG II)-induced hypertension was attenuated in mice with global (Nhe3/−) and Nhe3/− mice with transgenic rescue of the NHE3 gene selectively in small intestines (tgNhe3/−), suggesting an important role of NHE3 in the development of ANG II-dependent hypertension. In this study, we specifically tested whether the pharmacological inhibition of NHE3 mainly in the proximal tubules of the kidney attenuates ANG II-dependent hypertension induced by a low and slow pressor dose of ANG II supplemented with a high salt diet. Overall, 9 groups (n=5-12) of adult male C57BL/6J mice were infused with or without ANG II (500 μg/kg/day, i.p. via minipump) and supplemented with or without a 2% NaCl diet to slowly and moderately increase systolic blood pressure (SBP) in 2 weeks. ANG II alone increased SBP from 116 ± 2 mmHg to 140 ± 2 mmHg (p<0.01), and supplement of ANG II with a 2% NaCl diet further increased SBP to 147 ± 4 mmHg (p<0.05). Concurrent treatment with an orally active, absorbable NHE3 inhibitor AVE0657 (Sanofi-Aventis; 20 mg/kg/day, p.o.) significantly decreased SBP to 125 ± 4 mmHg in ANG II-infused mice (p<0.01), and to 134 ± 6 mmHg in ANG II-infused mice supplemented with 2% NaCl (p<0.01), respectively. Further treatment with AVE0657 and losartan, an AT1 receptor blocker (20 mg/kg/day, p.o.), completely normalize SBP in mice treated with ANG II and 2% NaCl to control (115 ± 5 mmHg, p<0.01). In the kidney, AVE0657 significantly increased 24h urinary Na+ excretion from 157.1 ± 6.7 to 207.7 ± 8.1 μmol/24h (p<0.01) without altering 24h urine excretion or SBP. Furthermore, AVE0657 did not significantly alter 24 h fecal Na+ excretion in non ANG II-infused (4.99 ± 0.37 μmol/24h, n.s.) or ANG II-infused mice (4.19 ± 0.67 μmol/24h, n.s.), compared with control (4.02 ± 0.20 μmol/24h, n.s.) or global Nhe3/− mice (50.8 ± 0.8 μmol/24h, p<0.01). Since small intestines in the gut and the proximal tubules of the kidney express the vast majority of NHE3 in the body, these results provide preclinical evidence and perspectives that orally absorbable NHE3 inhibitors may be pharmacologically beneficial to prevent and treat hypertension induced by ANG II and a high salt, mainly by inhibiting NHE3 in the proximal tubule of the kidney.

X.C. Li: None. H. Nguyen: None. J.L. Zhuo: None.

Funding: No

Funding Component:

012
Mitochondrial dysfunction has been implicated in various types of cardiovascular diseases which may involve overload and decompensation in mitochondrial quality/quantity control. However, limited mechanistic insight is available regarding the contribution and mechanism of mitochondrial quality control in hypertension. In the present study, we tested our hypothesis that enhancement of mitochondrial fission via Drp1 activation in vascular smooth muscle cells (VSMCs) is involved in hypertensive vascular remodeling. Rat aortic VSMCs pretreated with adenovirus encoding Drp1 siRNA (Ad-siDrp1) or control non-silencing RNA (100 moi) were stimulated with 100 nM angiotensin II (AngII) up to 72 h. 8 week old male C57/BL6 mice were infused with (1000 ng/kg/min) for 2 weeks with or without treatment of Drp1 inhibitor mdivi1 (25 mg/kg ip every other day). In VSMCs, AngII induced transient mitochondrial fission (max at 2-4 h assessed by mito-tracker staining) associated with Drp1 phosphorylation at Ser616 (10-30 min). Pretreatment of ad-siDrp1 (100 moi) or mdivi1 (5 μM) attenuated AngII-induced mitochondrial fission. Ad-siDrp1 or mdivi1 also attenuated AngII-induced enhancements of mitochondrial reactive oxygen species (ROS) generation, total cell protein, cell volume and extracellular collagen content. In mice, mdivi1 significantly suppressed vascular hypertrophy and perivascular fibrosis induced by AngII in aorta, heart and kidney. mdivi1 also inhibited AngII-induced left ventricular hypertrophy assessed by heart weight body weight ratio (mg/g: 7.8±0.9 vs 6.3±0.2 p<0.01) as well as by echocardiogram. However, mdivi1 did not affect hypertension induced by AngII assessed by telemetry (mean arterial pressure: sham 150±8 vs mdivi1 155±7 mmHg). KDEL and nitrotyrosine staining of the heart and kidney suggest attenuation of vascular ER stress and oxidative stress, respectively. In conclusion, this data suggests that Drp1-dependent mitochondrial fission contributes to AngII-induced cardiovascular remodeling independently of hypertension via enhancement of mitochondrial ROS and ER stress in target organs.

Genetic Ablation of Mas Receptor Impairs Mobilization of Bone Marrow Progenitor Cells

Angiotensin (Ang)-(1-7)/Mas receptor (MasR) pathway accelerates vascular repair in ischemic conditions partly by stimulating the mobilization of vascular reparative bone marrow progenitor cells (BMPCs) into blood circulation. This study tested if the endogenous MasR expression is required for the mobilization of BMPCs in response to ischemic injury. Hind limb ischemia (HLI) was induced in wild type (WT) or MasR knock out mice (MasR-KO) (in C57Bl/6J background). BMPCs in the blood circulation were quantitated by flow cytometric enumeration of Lineage−, Sca-1+ and cKit+ (LSK) cells in peripheral blood or by colony forming unit (CFU) assay. Subcutaneous osmotic pumps were used for continuous infusion of
Ang-(1-7) at the rate of 1 μg/kg/min for four weeks. In vitro migration of LSK cells in response to hypoxia-regulated factors, stromal-derived factor (SDF) or by vascular endothelial growth factor (VEGF) were determined. In WT mice, HLI stimulated mobilization of LSK cells that reached maximum by day 2 (110±11 cells/mL blood, n=6). Ang-(1-7)-treatment potentiated the peak mobilization (206±24 cells/mL blood, n=8, P<0.01 compared to the untreated). MasR-KO mice have reduced number of circulating LSKs (12±3 vs 43±9 per mL blood in WT, P<0.01, n=5) (CFUs/mL blood 28±5 vs 54±8 in WT, P<0.05, n=5). In MasR-KO mice, HLI did not induce mobilization, and blood flow recovery post-HLI was lower compared to WT (52±4% vs 89±6% in WT, P<0.001, n=5), both of which were not improved by treatment with Ang-(1-7). Number of bone marrow-resident LSK cells was higher in MasR-KO mice compared to WT. Migration induced by SDF (84±6% vs 160±8% in WT, P<0.001, n=5) or VEGF (97±4% vs 146±5% in WT, P<0.001, n=4) was decreased in MasR-KO. These results suggest that MasR deficiency causes impaired mobilization of BMPCs likely by decreasing their sensitivity to hypoxia-regulated factors. Therefore endogenous MasR expression is essential for ischemia-dependent mobilization of BMPCs.


Funding: Yes
Funding Component: National Center 014

Collecting Duct Specific Deletion of the (Pro)renin Receptor Modulates ENaC Expression

Nirupama Ramkumar, Deborah Stuart, Kai Song, Nikita Abraham, Shuping Wang, Univ of Utah, Salt Lake City, UT; Atsuhiro Ichihara, Tokyo Women’s Medical Univ, Tokyo, Japan; Donald E Kohan, Univ of Utah, Salt Lake City, UT

The renal tubular (pro)renin receptor (PRR) has been shown to modulate water balance, blood pressure and Na+ homeostasis. We recently reported that inducible nephron wide deletion of the PRR results in Na+ wasting, reduced epithelial Na+ channel (ENaC) expression in the kidney and attenuated hypertensive response to angiotensin-II (Ang-II) infusion. In this study, we examined the effects of PRR deletion in collecting duct (CD) specific mouse models targeting either the principal cells (PC) or intercalated cells (IC). PC-specific PRR knockout (KO) mice were obtained by crossing floxed PRR mice with mice harboring AQP-2 Cre recombinase. Compared to floxed mice, PC specific KO PRR mice had no differences in PRR immunostaining but had 50% reduction in PRR mRNA in micro-dissected cortical CDs. No differences in blood pressure were observed between the two groups at baseline or following Ang-II infusion at 600 ng/kg/min. Similarly, plasma renin concentration and renal expression of ENaC protein isoforms were comparable between the two groups. To achieve IC-specific PRR deletion, floxed PRR mice were bred with mice expressing B-1 Cre recombinase. Compared to floxed controls, IC-specific PRR KO mice were smaller (KO body weight: 5.9 ± 1.3 g vs controls: 11.1± 1.2 g) and did not survive beyond 30 days after birth. IC-specific PRR KO mice also demonstrated marked reduction in renal medullary PRR immunostaining along with decreased renal expression of ENaC-α protein (50% reduction compared to controls), similar to the findings in nephron wide deletion of PRR. Taken together, these findings suggest that IC specific deletion of PRR but not PC-specific deletion modulates renal ENaC expression. Further studies evaluating ENaC activity in isolated cortical CDs from PC and IC specific PRR KO mice will help
delineate the functional role of CD PRR in Na+ homeostasis.


Funding: No

Funding Component:

015

**Immunosuppression Attenuates Intrarenal Angiotensinogen Augmentation in Angiotensin II Dependent Hypertension**

**Ryousuke Satou**, Dept of Physiology and Hypertension and Renal Ctr of Excellence, Tulane Univ Sch of Med, New Orleans, LA; Martha G Franco, Depts of Nephrology and Pathology, Insto Nacional de Cardiologia, Mexico City, Mexico; Akemi Katsurada, Kayoko Miyata, L G Navar, Dept of Physiology and Hypertension and Renal Ctr of Excellence, Tulane Univ Sch of Med, New Orleans, LA

Augmented intrarenal angiotensinogen (AGT) is a critical contributor to activation of intrarenal renin-angiotensin system (RAS) leading to the development of hypertension and associated kidney injury. It has been shown that treatment with mycophenolate mofetil (MMF), an immunosuppressive drug, mitigates the increased intrarenal angiotensin (Ang) II levels and blood pressure in hypertensive animal models, suggesting that an activated immune system mediates intrarenal RAS activation and consequent hypertension. Associated macrophage (МФ) infiltration augments pro-inflammatory cytokine levels including interleukin-6 (IL-6), which plays a crucial role in augmentation of AGT expression in cultured renal proximal tubular cells. Accordingly, this study was performed to establish pathophysiological relevance for the effects of stimulated МФ and IL-6 on intrarenal AGT augmentation in Ang II-dependent hypertension. Ang II (80 ng/min) was infused with/without daily MMF administration (50 ng/kg) to Sprague-Dawley rats for 2 weeks. Mean arterial pressure (MAP) in Ang II infused rats was slightly higher (169.7±6.1 mmHg) than MAP in Ang II+MMF group (154.7±2.0 mmHg) which was not statistically different than in control group. The augmentation of urinary AGT and urinary protein by Ang II infusion was attenuated by MMF treatment (AGT, control: 89.3±25.2, Ang II: 1,194±305.1, and Ang II+MMF: 389±192.0 ng/day). Importantly, the augmentation of urinary AGT by Ang II infusion was observed before the onset of proteinuria. Urinary 8-isoprostane levels were not altered by Ang II and/or MMF during the 2-week treatments. MMF treatment suppressed Ang II-induced renal МФ infiltration and IL-6 elevation (IL-6 mRNA, Ang II: 32.4±7.5 and Ang II+MMF: 3.6±1.7, ratio to control). qRT-PCR, western blot and immunohistochemistry revealed elevated intrarenal AGT mRNA and protein levels in Ang II infused rats which were normalized by the MMF treatment (AGT mRNA, Ang II: 2.5±0.2 and Ang II+MMF: 1.5±0.1, ratio to control). These results indicate that stimulated IL-6 production in infiltrated МФ contributes to intrarenal AGT augmentation in early stages of Ang II-dependent hypertension, which contributes to the development of kidney injury.


Funding: No

Funding Component:

016

**Cells Programmed for the Renin Phenotype Contribute to Vascular Pathology in Renin Deficient Mice**
Masafumi Oka, Silvia Medrano, Maria Luisa S Sequeira-Lopez, R Ariel Gomez, Univ of Virginia, Charlottesville, VA

Deletions of the renin-angiotensin system genes or pharmacological inhibition in early life result in a distinctive renal pathology: concentric and disorganized intra-renal arteriolar thickening. The origin and distribution of the cells contributing to the arterial disease are not known. Because the arteriolar thickening disappears with ablation of renin cells, we hypothesized that renin cell precursors contribute to the arterial pathology. To reveal the origin and distribution of the cells responsible for the arterial thickening we generated several mouse lines for fate tracing and also stained for cell identity specific proteins. Kidneys from \( \text{Ren1c-/-} \) (n=6) and \( \text{Ren1c+/} \) (n=6) mice were immunostained for renin, αSMA and PECAM1. Arterial wall thickness was measured using a light microscope and the Leica MM AF® version1.5 software. Renin cells (unable to produce renin because of the knock out) were identified using \( \text{Ren1c-/-; Ren1c-YFP} \) mice, where the yellow fluorescent protein is expressed by the \( \text{Ren1c-YFP} \) transgene designed to label all cells with an active renin promoter. In addition, we tracked the expression and distribution of aldo-keto reductase 1b7, AKR1b7, which mark cells programmed for the renin phenotype even when renin is absent. As expected, \( \text{Ren1c-/-} \) kidneys showed no renin and thicker intra-renal arteries (Arterioles: \( \text{Ren1c+/-} \); 8.26 ± 2.5 μm vs. \( \text{Ren1c-/-} \); 14.3 ± 3.8 μm, \( P<0.0001 \), larger arteries: \( \text{Ren1c+/-} \); 29.2 ± 11.1 μm vs. \( \text{Ren1c-/-} \); 42.1 ± 11.1 μm, \( P<0.0001 \) AKR1b7+ and YFP+ cells were retained and observed throughout the renal arterioles. To investigate the fate and distribution of cells from the renin lineage, we used \( \text{Ren1c-Cre} \) and \( \text{R26R.LacZ or mT/mG} \) reporter mice (6 knock out and 6 control mice per strain). Cells from the renin lineage surrounded arterioles and persisted within larger arterial walls whereas PECAM1+ endothelial cells did not contribute to the arterial wall thickening. In control mice, renin cells were confined to the juxtaglomerular area. We conclude that precursor cells programmed for the renin phenotype maintain their molecular program and together with vascular smooth muscle cells contribute to nephrovascular disease.

M. Oka: None. S. Medrano: None. M.S. Sequeira-Lopez: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; DK091330 and DK096373. R.A. Gomez: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL066242 and DK096373.

Funding: No
Funding Component:
017

Optogenetic Activation of OVLT Neurons Stimulates Water Intake and Produces a Sympathetically-Mediated Increase in Arterial Blood Pressure

Sean D Stocker, Sarah S Simmonds, Penn State Univ Coll of Med, Hershey, PA

The organum vasculosum of the lamina terminalis (OVLT) plays a pivotal role in body fluid homeostasis and arterial blood pressure (ABP) regulation. The OVLT lacks a complete blood-brain-barrier and responds to an array of circulating factors such as NaCl and angiotensin II. Lesion of the anteroventral third ventricular region which includes the OVLT attenuates or reverses several forms of salt-sensitive hypertension. However, there is limited
evidence to demonstrate that direct activation of OVLT neurons alters body fluid homeostasis or elevates ABP. To address this question, Male-Sprague-Dawley rats (300-350 g) received an injection of rAAV9-CamKII-hChR2(H134R)-EYFP (10^{12} particles/mL, 200nL) into the OVLT. A fiber optic cannula (200µm) was implanted 300µm dorsal to OVLT. Approximately 2-3 week later, optogenetic activation of OVLT neurons (10ms pulse, 50% duty cycle, 30 min) produced frequency-dependent increases in water intake (1Hz: 1.0±0.5mL; 5Hz: 4.2±0.6mL; 10Hz: 8.0±1.8; 20Hz: 10.2±2.1mL, n=4, P<0.05). In separate experiments, optogenetic activation of OVLT neurons produced a frequency-dependent increase in mean ABP (1Hz: 1±1 mmHg; 5Hz: 3±1mmHg; 10Hz: 7±1mmHg; 20Hz: 13±1mmHg, n=4, P<0.05) and heart rate (1Hz: 3±6 bpm; 5Hz: 15±5bpm; 10Hz: 40±12 bpm; 20Hz: 62±14bpm, n=4, P<0.05). Pretreatment with the vasopressin antagonist Manning Compound (10ug/kg, IV) did not affect these responses. However, pretreatment with the ganglionic blocker chlorisondamine (5mg/kg, IV) abolished the pressor (20Hz: 1±1 mmHg, P<0.01) and tachycardic (20Hz: 4±7 bpm, P<0.05) responses to activation of OVLT neurons. Finally, in vivo single-unit recordings demonstrate that optogenetic activation produced frequency-dependent increases in cell discharge of OVLT neurons responsive to either intracarotid injection of hypertonic NaCl (0.3M NaCl, 50µL over 10 s, n=6) or angiotensin II (100ng over 10s, n=3). Collectively, these data provide evidence that direct activation of OVLT neurons stimulates thirst and produces a sympathetically-mediated increase in ABP.

S.D. Stocker: None. S.S. Simmonds: None.

Funding: Yes
Funding Component: National Center 018

Afferent-targeted Renal Denervation Attenuates Established Deoxycorticosterone-salt Hypertension: A Central Role for Renal Afferent Nerves in Hypertension?

Christopher T Banek, Dusty Van Helden, Ninitha Asirvatham-Jeyaraj, John W Osborn, Univ of Minnesota, Minneapolis, MN

Cardiovascular disease (CVD) remains the most pervasive cause of death worldwide. High arterial pressure, or hypertension (HTN), is the highest risk factor for CVD morbidity and mortality. Increased peripheral and renal sympathetic nerve activity (SNA) is hypothesized to be a primary contributor to HTN etiology. Moreover, recent clinical and experimental studies show total renal denervation (T-RDNx), may reverse the HTN; however, the contribution of afferent and efferent renal nerves in this effect is unknown. We have recently reported T-RDNx and afferent-specific denervation (A-RDNx) identically attenuated the development of deoxycorticosterone acetate (DOCA)-salt hypertension. However, the efficacy of T-RDNx and A-RDNx to reverse the established phase of this model of HTN is unknown. Therefore, the present study tested the hypothesis that A-RDNx and T-RDNx would similarly decrease the mean arterial pressure (MAP) in DOCA-salt rats with established HTN. Twenty-four male Sprague Dawley rats (275-300g) instrumented with radiotelemeters were administered DOCA (100mg, s.c.) and 0.9% saline to drink ad libitum for 35 days. On day 21 of DOCA-salt, rats underwent T-RDNx (n=9), A-RDNx (n=9), or sham (n=6) treatments. MAP was monitored for an additional 14 days. Neurogenic pressor activity (NPA) was assessed 14 days after treatment by measuring the MAP response to acute ganglionic blockade (hexamethonium, 30mg/kg, i.p.). Data was analyzed with a one-way ANOVA with Bonferroni post-hoc test.
(α=0.05). Data presented as mean ± SEM. MAP was similar across all groups prior to treatment on Day 21 of DOCA-salt (Sham: 165±6; T-RDNx: 164±3; A-RDNx: 162±7mmHg). Whereas Sham had no effect (-2±3) on MAP 14 days after treatment, both RDNx treatments decreased MAP by approximately 20 mmHg (T-RDNx - 18±8; A-RDNx -22±5). NPA 14 days after treatment in Sham rats was -97±12mmHg. This response was reduced by nearly half in both T-RDNx (-50±9mmHg) and A-RDNx (-48±4mmHg) groups. We conclude from these findings that: 1) RDNx is effective in treating the established phase of DOCA-salt hypertension, 2) the MAP response to RDNx is mediated by ablation of afferent renal nerves, and 3) the antihypertensive response to RDNx is mediated by a decrease global neurogenic pressor activity.


Funding Component:

019

Evidence that Remodeling of Insular Cortex Neurovascular Unit Contributes to Hypertension-related Sympathoexcitation

Fernanda Ribeiro Marins, Univ Federal de Minas Gerais, Belo Horizonte, Brazil; Jennifer A Iddings, Augusta Univ, Augusta, GA; Marco Antonio Fontes, Univ Federal de Minas Gerais, Belo Horizonte, Brazil; Jessica A Filosa, Augusta Univ, Augusta, GA

Introduction and Hypothesis: The intermediate region of the posterior insular cortex (intermediate IC) mediates sympathoexcitatory responses to the heart and kidneys. Previous evidence indicates that hypertension alters both structure and function of neurons, blood vessels, astrocytes and microglia, disrupting the architecture of the neurovascular unit (NVU) in specific brain regions. Thus, the goal of this study is to evaluate the functional and anatomical integrity of the NVU in the intermediate IC during hypertension using in vivo and in situ experiments in male hypertensive (SHR) and normotensive (WKY) rats. Methods: Under urethane anesthesia, NMDA microinjection (0.2mM/100nL) was performed in the intermediate IC with simultaneous recording of renal sympathetic nerve activity (RSNA), heart rate (HR) and mean arterial pressure (MAP). NVU structure was investigated by immunofluorescence for NMDA receptors (NR1, NeuN and TOTO), blood vessels (perfused with 70kDa FITC-dextran), astrocytes (GFAP) and microglia (Iba1). Results: NMDA injections into intermediate IC of SHR (n=4) evoked higher amplitude responses of RSNA (∆= WKY 26 ± 1.5 vs. SHR 44 ± 4.1 % of baseline, P=0.006), MAP (∆= WKY 9 ± 1.8 vs. SHR 19 ± 2.2 mmHg, P=0.017) and HR (∆=WKY 40 ± 2.5 vs. SHR 54 ± 4.9 bpm, P =0.044). Immunofluorescence data of the intermediate IC of SHR showed increased NMDA receptor density (WKY 16.67± 1.05% vs. SHR 24.17± 1.68%, n=6, P=0.003). Vascular density (WKY 1.73± 0.13% vs. SHR 2.52± 0.27%, n=10, P=0.015), branch and end-point number were also increased suggesting angiogenesis at the IC of SHR. Additionally, IC of SHR presented greater GFAP immunoreactivity (WKY 9.37± 2.28% vs. SHR 19.51± 3.52%, n=13, P=0.023) and increased contact between astrocyte processes and the vasculature (∆= 12.8%, n=13, P=0.015). Skeleton analysis indicated enhanced microglia activation in IC of SHR (reduced number of branches, junctions, end-points and process length, n=13), suggesting an inflammatory process in this region. Conclusions: These findings suggest that the neurogenic origin of hypertension in SHR is associated with marked alterations to NVU structure within the IC contributing to
enhanced NMDA-mediated sympathoexcitatory responses and maintenance of hypertension.

**F.R. Marins:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brazil (CNPQ 306000/2013-0), Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG), CAPES (99999.003909/2015-08 Programa PDSE), INCT Nanobiofar, National Heart, Lung, and Blood Institute (NHLBI) of the NIH (R01 HL089067-02 to JAF).

**J.A. Iddings:** None.

**M.A.P. Fontes:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brazil (CNPQ 306000/2013-0), Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG), INCT Nanobiofar.

**J.A. Filosa:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; National Heart, Lung, and Blood Institute (NHLBI) of the NIH (R01 HL089067-02 to JAF).

**Funding:** No

**Funding Component:**

**020**

**Physiological Significance of Angiotensin AT_{1A} Receptors in Vasopressin-producing Cells of the Supraoptic Nucleus**

Danny W Linggongegoro, Jeremy A Sandgren, Kristin E Claflin, Katherine J Perschbacher, Jonathan Ni, Nicole A Pearson, Gary L Pierce, Mark K Santillan, Justin L Grobe, Univ of Iowa, Iowa City, IA

Low-renin and salt-sensitive forms of hypertension are characterized by elevated activity of the brain renin-angiotensin system and secretion of arginine vasopressin (AVP). While angiotensin in the brain is a known stimulant of AVP secretion through its AT_{1} receptor, the localization of relevant AT_{1} receptors remains unclear. We tested whether AT_{1A} receptors localized to AVP-producing cells are important for AVP secretion. To examine AVP and AT_{1A} co-localization, mice expressing Cre-recombinase via the AVP gene (AVP-Cre) were bred with mice expressing a conditional red fluorescent ROSA-stop^{flox}-tdTomato construct and GFP via an AT_{1A} BAC transgene. Dual-fluorescent cells were detected in supraoptic nuclei (SON) but not paraventricular nuclei. Mice lacking AT_{1A} specifically in AVP-producing cells (AT_{1A}^{AVP-KO}) were then generated by breeding AVP-Cre mice with mice harboring a conditional endogenous AT_{1A} gene. AT_{1A}^{AVP-KO} mice exhibited normal serum (littermate n=13, 353±69 vs AT_{1A}^{AVP-KO} n=7, 207±37 pg/mL, p=NS) and urine (n=26, 145±33 vs n=11, 170±54 pg/mL, p=NS) copeptin (the stable C-terminal fragment of AVP) as well as hematocrit (n=14, 46.3±0.7 vs n=7, 47.5±1.3 %, p=NS) despite increased serum osmolality (n=33, 324±1.3 vs n=19, 330±1.6 mOsm/kg, p<0.01), supporting a role for AT_{1A} in AVP-producing cells in modulating the osmotic control of AVP release. Systolic blood pressure (SBP) (n=18, 109±1.3 vs n=5, 107±1.2 mmHg), urine volume (n=27, 1.1±0.1 vs n=12, 0.9±0.2 mL/d), and fluid intake (n=27, 4.0±0.2 vs n=12, 3.9±0.2 mL/d) were all normal (p=NS) in AT_{1A}^{AVP-KO} mice. Two-bottle choice between water and escalating concentrations of NaCl uncovered minor alterations in sodium intake behavior. Serum osmolality (n=22, 336±2 vs n=9, 333±3 mOsm/kg), SBP (n=23, +10.4±2.1 vs n=8, +12.9±2.0 mmHg), urine output (n=23, +12.7±0.8 vs n=9, +12.7±1.5 g/day), and fluid intake (n=23, +16.2±1.3 vs n=9, +14.8±2.5 mL/day) all increased normally (p=NS) in
response to deoxycorticosterone acetate (DOCA)-salt treatment. Collectively these data support a role for AT_{1A} receptors, localized specifically to AVP-expressing cells of the SON, in the normal osmotic control of AVP secretion.

**D.W. Linggomegoro:** None.  **J.A. Sandgren:** None.  **K.E. Claflin:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA.  **K.J. Perschbacher:** None.  **J. Ni:** None.  **N.A. Pearson:** None.  **G.L. Pierce:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA.  **M.K. Santillan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA.  **J.L. Grobe:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA.

Funding: Yes  
Funding Component: National Center  
021

**Macrophage-dependent Impairment of the Alpha 2-adrenergic Receptor Occurs in DOCA-salt but Not Obesity-associated Hypertension**

Ryan Mui, Roxanne Fernandes, Hannah Garver, Gregory Fink, Hui Xu, James Galligan, Michigan State Univ, East Lansing, MI

Inflammation and increased sympathetic activity contribute to hypertension. Alpha 2-adrenergic receptor (α2AR) activation decreases norepinephrine (NE) release from sympathetic nerves by inhibiting Ca^{2+} channels. We hypothesized that macrophage infiltration into mesenteric arteries (MA) impairs α2AR in salt-sensitive and obesity-associated hypertensive rats. Uniphrectomized Sprague Dawley (SD) rats were given water (SHAM) or 200mg/kg DOCA (pellet, sc) and water containing 1% NaCl, 0.2% KCl (DOCA) for 4 weeks. A high fat diet (HFD, 60% kcal from fat, 0.33% NaCl, 1% K^+) or normal fat diet (NFD, 10% kcal from fat, 0.24% NaCl, 0.36% K^+) was given to a second group of SD rats for 20 weeks and to Dahl salt-sensitive (SS) rats for 24-26 weeks after weaning (3 weeks). Immunohistochemistry for CD163 (macrophage marker) was used to count macrophages in MA. Whole-cell patch clamp was used on dissociated celiac ganglion neurons to evaluate α2AR-mediated Ca^{2+} current inhibition with NE (1 μM). Liposome-encapsulated clodronate (Clod) was used to deplete rats of macrophages. Plasma aldosterone levels were assessed by ELISA. Summary data is provided in Table 1. Systolic blood pressure was higher in DOCA vs SHAM and HFD vs NFD rats. Vascular macrophage number increased in DOCA vs SHAM but not in HFD vs NFD rats. NE inhibited Ca^{2+} current to a greater degree in neurons of SHAM vs DOCA but not NFD vs HFD rats. Clodronate reduced vascular macrophages in all rats and preserved α2AR-mediated inhibition of Ca^{2+} current in DOCA rats. HFD did not affect plasma aldosterone levels. Therefore, we conclude that macrophage-associated impairment of α2AR may only occur in states of mineralocorticoid and salt excess.

<table>
<thead>
<tr>
<th>Systolic Pressure (mmHg)</th>
<th>Macrophages per 0.1 mm^2</th>
<th>%Ca^{2+} Current Inhibition</th>
<th>Aldosterone [ng/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM (n=10)</td>
<td>131 ± 6</td>
<td>91 ± 3</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>DOCA (n=10)</td>
<td>134 ± 8</td>
<td>93 ± 3</td>
<td>59 ± 4</td>
</tr>
<tr>
<td>SHAM-Clod (n=6)</td>
<td>131 ± 6</td>
<td>84 ± 3</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>DOCA-Clod (n=6)</td>
<td>136 ± 6</td>
<td>83 ± 3</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>SD NFD (n=5)</td>
<td>137 ± 6</td>
<td>73 ± 1</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>SD Clod (n=6)</td>
<td>138 ± 6</td>
<td>61 ± 1</td>
<td>44 ± 3</td>
</tr>
</tbody>
</table>

All values are presented as mean ± SE  
*p<0.05, statistically significant (t-test)
Blood Pressure-Lowering Effect of Local Passive Heat in Autonomic Failure Patients with Supine Hypertension

Luis E Okamoto, Jorge E Celedonio, Alfredo Gamboa, Cyndya A Shibao, Satish R Raj, Andre Diedrich, Sachin Paranjape, Bonnie K Black, David Robertson, Vanderbilt Univ Medical Ctr, Nashville, TN; Craig C Crandall, Univ of Texas Southwestern Medical Ctr, Dallas, TX; Italo Biaggioni, Vanderbilt Univ Medical Ctr, Nashville, TN

Primary autonomic failure (AF) is characterized by disabling orthostatic hypotension that is acutely worsened by environmental heat. Given that about half of AF patients have paradoxical supine hypertension, we hypothesized that controlled local passive heat would lower supine blood pressure (BP) in these patients. Fourteen AF patients with supine hypertension (age 71±2 years, 9 men, systolic BP 172±6 mmHg) were randomized to receive passive heat (40-42°C, commercial heating pad over abdomen and pelvis) and sham control for up to 2 hours in a 2-day crossover study. Hemodynamic parameters and core body and skin temperatures were measured in the supine position. The heating pad increased abdominal skin temperature to 40.8±0.4°C and 40.1±0.3°C after 1 and 2 hours of passive heat (vs 35.2±0.2°C and 35.1±0.4°C in sham controls). Core body temperature increased after 1 hour (by 0.2±0.1°C [to 36.9±0.8°C] vs 0.0°C [36.7±0.1°C] in sham controls; P=0.04) and 2 hours (by 0.4±0.1°C [to 37.2±0.1°C] vs 0.1±0.03°C [to 36.8±0.1°C] in sham controls; P=0.04). Systolic BP decreased during heat stress compared to sham control (P<0.01 by mixed-effects model) with a maximal reduction at 1.7 hours of -26±5 mmHg (Figure). This BP drop was due to a decrease in cardiac output (-30±5% vs sham -5±3%; P=0.02) and stroke volume (-29±5% vs sham -6±3%; P<0.01). Systemic vascular resistance and heart rate were similar in both groups. In conclusion, low levels of local passive heat had a BP-lowering effect in AF patients with supine hypertension presumably due to an uncompensated decrease in central blood volume. The therapeutic application of this approach needs to be addressed in future studies.
Angiotensin-(1-7) Reduces Doxorubicin-Induced Cardiac Fibrosis


Doxorubicin (Dox) is a commonly used and effective chemotherapeutic agent for childhood leukemias and sarcomas. However, Dox administration often results in cumulative dose-dependent cardiotoxicity that manifests as cardiomyopathy with marked fibrosis that leads to reduced cardiovascular function and heart failure. Consequently, there is a need for adjunct therapies to reduce Dox-induced cardiotoxicity and enhance long-term quality-of-life in cancer patients, especially in pediatric patients. Angiotensin-(1-7) [Ang-(1-7)] is an endogenous peptide hormone of the renin-angiotensin system with cardioprotective properties; studies by us and others showed that Ang-(1-7) reduces cardiac fibrosis and improves cardiac function in various animal models. In this study, we investigated whether adjunct Ang-(1-7) attenuates cardiotoxicity resulting from an acute (6 week) exposure of juvenile Sprague-Dawley rats (male, n = 8-10) to Dox (22 mg/kg). Dox treatment reduced body mass, cardiac weight and cardiomyocyte size, while Ang-(1-7) co-administration had no effect on these changes. However, co-administration of Ang-(1-7) prevented Dox-mediated increases in cardiac fibrosis (interstitial fibrosis: 1.4 ± 0.1% to 2.5 ± 0.3%; perivascular fibrosis: 15.8 ± 1.4% to 23.2 ± 1.6%; p < 0.01) and coronary vessel hypertrophy (media-to-lumen ratio: 2.4 ± 0.2 to 3.3 ± 0.3; p < 0.05). Administration of Dox significantly increased E/E' by 47%, a marker of diastolic dysfunction, and pulse wave velocity by 74%, as a measure of arterial stiffness, quantified using a Vevo 2100 small animal ultrasound system; co-administration of Ang-(1-7) prevented the Dox-induced increases in diastolic dysfunction and arterial stiffness. In contrast, Ang-(1-7) had no effect on the Dox-mediated reductions in ventricular diameter, parasternal wall thickness, stroke volume and cardiac output. The anti-fibrotic and anti-hypertrophic effects of Ang-(1-7) may account for the improvement in diastolic and arterial function. Collectively, these results suggest that adjunct Ang-(1-7) may attenuate cardiac fibrosis and toxicity induced by Dox administration.

O. Rahimi: None. E. Tallant: None. P.E. Gallagher: None.

Funding: No
Funding Component:

PGC-1α is a Critical Activator of HO-1 That Protects Against Cardiomyopathy in Diabetic Mice Through Recruitment of Mitochondrial Fusion Proteins and Function

Jian Cao, First Dept of Geriatric Cardiology, Chinese PLA General Hosp, Beijing, China; John A. McClung, Dept of Cardiology, New York Medical Coll, Valhalla, NY; Maayan Waldman, Leevie Heart Ctr Sheba Medical Ctr, Tel Hashomer and Sackler Sch of Med, Cardiac Res Lab, Felsenstein Medical Res Inst, Tel-Aviv Univ, Petach-Tikva, Israel; Shailendra P. Singh, Joseph Schragenheim, Dept of Pharmacology, New York Medical Coll, Valhalla, NY; Michael Arad, Cardiac Res Lab, Felsenstein Medical Res Inst, Tel-Aviv Univ, Petach-Tikva, Israel; Edith
Introduction: Diabetes mellitus type 2 (DM2) is associated with cardiovascular complications, which are characterized by increased oxidative stress (ROS) and inhibition of anti-oxidant genes such as heme oxygenase (HO-1), Super oxide dismutase (SOD2) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). The latter that controls mitochondrial biogenesis, oxidative metabolism, and increased degradation of epoxyeicosatrienoic acids (EETs). Inhibition of these genes leads to increased myocardial stiffness and the development of cardiac hypertrophy and diastolic dysfunction. Aim: To assess whether PGC-1α plays a significant role in the development of diabetic cardiomyopathy in chronically obese mice. Methods: Leptin resistant (db/db) mice develop cardiomyopathy at the age of 5-6 month. Mice were treated with the EET agonist (EET-A) and with either lentivirus (Ln)- PGC-1α (Sh) or EET-A-Ln-PGC-1α scrambled for 3 additional months. Results: db mice exhibited impaired glucose tolerance, increased fasting blood glucose (366±21.9mg/dL vs.112±9.2 mg/dL, p<0.004), decreased oxygen consumption (VO₂) (25.09±1.1ml/min vs. 55.37±5.92ml/min, p<0.002) and heart weight (0.17±0.02g vs. 0.12±0.006g, p<0.05) compared to WT mice. EET agonists treatments improved fasting blood glucose (366±21.9mg/dL vs. 134±18.4 mg/dL, p<0.007) and oxygen consumption (25.09±1.1ml/min vs. 33.7±3.75ml/min, p<0.018), and reduced heart weight (0.17±0.02g vs. 0.13±0.02g, p<0.0026). HO-1 levels were increased in cardiac tissue 22-fold (p<0.016). The beneficial effects of EET were reversed inhibition of PGC-1α in the EET-A-Ln PGC-1α (Sh) not in EET-A-Ln-PGC-1α scrambled group. The inhibition of PGC-1α (52% reduction, p<0.022) resulted in a reduction in the beneficial effects of EET-A as manifested by weight gain, the development of severe dilated cardiomyopathy and the attenuation of HO-1 and SOD2 (90% and 76% reduction respectively, p<0.02) and a decrease in mitochondrial fusion proteins Mfn1, 2 and OPa1.

Conclusion: EET mediated restoration of mitochondrial function by PGC1α is essential for the enhancement of HO-1-myocyte contraction and the prevention of LV dysfunction in chronic obesity.

Funding: No

Funding Component:

025

AMP-activated Protein Kinase Mediates Pressure Overload-induced Hypertrophy

Hypertension is a major risk factor of death and disability from heart and vascular diseases. Heart hypertrophy caused by pressure overload is characterized by the activation of adenosine monophosphate-activated protein kinase (AMPK), which is the major energy sensor in the heart. However, the anti-inflammatory effect of AMPK has not been investigated in the hypertrophy model. Activated protein C (APC) is a vitamin-K dependent plasma serine protease which inhibits blood clotting, and APC is an
endogenous AMPK agonist. This study was designed to examine the role of AMPK in hypertrophy and the underlying mechanisms by which APC inhibits heart hypotrophy by pressure overload. We hypothesize that AMPK play a role in preventing heart from hypertrophy induced by pressure overload. APC could inhibit high blood pressure-induced hypertrophy via activation of AMPK signaling pathway. Wild-type (WT) and AMPK-kinase dead (KD) transgenic mice were subjected to transverse aortic constriction (TAC) surgery. Echocardiography was performed to evaluate the heart function, and histology staining revealed the morphological changes. Real-time PCR and western blotting were used to detect the signaling changes of both mRNA and protein expression levels. There is no phenotype difference between WT and AMPK-KD mice under normal physiological conditions. However after 4 weeks of TAC surgery, AMPK-KD mice demonstrated significantly bigger heart than WT mice (p<0.05), and the cardiac functions measured by echocardiography in AMPK-KD hearts were significantly impaired as compared with WT hearts (p<0.05). The immunohistochemical staining showed that the increased macrophage infiltration and reactive oxygen species (ROS) including activated p66shc, 4-HNE and ERK were observed in the AMPK-KD hearts after 4 weeks of TAC surgery (all p<0.05 versus WT hearts). APC administration significantly attenuated hypertrophy and fibrosis caused by pressure overload, and macrophage infiltration and p66shc activation were also inhibited by APC treatment. Therefore, Cardiac AMPK deficiency aggravates hypertrophy caused by pressure overload. AMPK activator APC could be a therapeutic drug for treatment of hypertrophy by high blood pressure.

Funding: Yes
Funding Component: National Center 026

Role of Tank in the Regulation of Pathological Cardiac Hypertrophy

Hongliang Li, Peng Zhang, Wuhan Univ, Wuhan, China

TRAF associated NF-κB activator (TANK) is adaptor protein which was identified as a negative regulator of TRAF-, TBK1- and IKKi-mediated signal transduction through its interaction with them. Besides its important roles in the regulation of immune response, it has been reported that TANK contributes to the development of autoimmune nephritis and osteoclastogenesis. However, its functions in cardiovascular diseases especially cardiac hypertrophy is largely unknown. In the present study, we interestingly observed that TANK expression is increased by 240% in human hypertrophic cardiomyopathy (HCM) tissue and 320% in mouse hypertrophic heart after aortic banding (AB), indicating that TANK may be involved in the pathogenesis of this diseases. Subsequently, cardiac-specific TANK knockout (TANK-KO) and transgenic(TANK-TG)mice were generated and subjected to AB for 4 to 8 weeks. Our results demonstrated that TANK deficiency prevented against cardiac hypertrophy and fibrosis induced by pressure overload, as evidenced by that the cardiomyocytes enlargement and fibrosis formation was reduced by about 34% and 43% compared with WT mice, respectively. Conversely, TANK-TG mice showed an aggravated effect on cardiac hypertrophy in response to pressure overload with 36% and 47% increase of cardiomyocytes enlargement and fibrosis formation compared with non-transgenic mice. More importantly, in vitro experiments further revealed that TANK overexpression which was mediated by
adenovirus in the cardiomyocytes dramatically increased the cell size and the expression of hypertrophic markers, whereas TANK knockdown had an opposite function. Mechanistically, we discovered that AKT signaling was activated (230%) in the hearts of TANK-TG mice, while being greatly reduced in TNAK-KO hearts after aortic banding. Moreover, blocking AKT/GSK3β signaling with a pharmacological AKT inhibitor reversed cardiac dysfunction of TANK-TG mice. Collectively, our data show that TNAX acts as a novel regulator of pathological cardiac hypertrophy and may be a promising therapeutic target.

H. Li: None. P. Zhang: None.

Funding: No

Funding Component: 027

Participation of Cardiac Thyrotroping Releasing Hormone (cTRH) in the Doxorrubicin (D) Induced Cardiotoxicity in Mice

Ludmila S Peres Diaz, Mariano L Schuman, Maia Aisicovich, Maria S Landa, Silvia I García, Inst of Medical Res A. Lanari, CABA, Argentina

We have demonstrated TRH hyperactivity in the hypertrophied Left ventricle (LV) of SHR. Its specific inhibition attenuates hypertrophy development in spite of the significant higher pressure observed (Schuman M, Hypertension 2011). LV-TRH over-expression induces several features of the hypertrophied heart, including increases in the apoptotic index Bax/Bcl2, and the activated caspase 3 observed by immunohistochemistry (Schuman M, AJPHEC 2014). In addition, we have found that TRH expression was induced by D in primary cultures of cardiac cells. Based on these results we hypothesized that cTRH could participate in the D cardiotoxicity effects. Indeed, we used C57 adult males (n=10) with a single D or saline injection (200uL, 10 mg/kg ip) which previously (24 h, under anesthesia) received an intracardiac injection of a specific TRH-siRNA to inhibit LV-TRH expression or scrambled Con-siRNA. Mice were sacrificed 2, 4 and 7 days post D injection and body weight was measured. Genes expression was measured by real time PCR and protein by immunohistochemistry (ANOVA and Tukey test). Body weight showed a mild but not significant decrease in D treated animals. D significantly increased TRH gene expression and TRH protein content reaching the maximum at 7 days post injection (2d: 145%, 4d: 190% and 7d: 250%) (p & 0.05), which were not observed in the groups with D+TRH-siRNA indicating the effectiveness of the specific TRH inhibition. Also at this time TRH inhibition attenuates (p&0.05) D-induced increase in the apoptotic index Bax/Bcl2 and the augmented activated caspase 3 content pointing out the participation of the cardiac TRH in the D-induced apoptosis. Similar results were observed with hypertrophic and fibrotic markers gene expression (BNP, BMHC and col III) which showed a significant increase (p& 0.05) only in the groups with D and the intact TRH system (D+Con-siRNA). Fibrosis results were confirmed by Sirius Red and Masson techniques.

On the whole, we demonstrated for the first time that LV-TRH system is required for both, Doxorubicin induction of apoptosis and consecutively hypertrophy and fibrosis in the mouse heart. Even more, we found that cTRH inhibition attenuates doxorubicin induced damage suggesting a novel mechanisms in the cardiotoxicity injury.

L.S. Peres Diaz: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PIP 11220120100491 CONICET-ARGENTINA. M.L. Schuman: B. Research Grant (includes principal
Effects of N-acetyl-eryld-aspartyl-lysyl-proline on Inflammatory Cells During the Acute Stage of Myocardial Infarction

Pablo Nakagawa, Ginette Bordcoch de Martino, Martin D’Ambrosio, Xu Jiang, Oscar Carretero, Henry Ford Hosp, Detroit, MI

Background: The natural peptide N-Acetyl-Seryl-Aspartyl-Lysyl-Proline (Ac-SDKP) decreases inflammation in chronic diseases such as hypertension and heart failure. The effects of Ac-SDKP on acute inflammatory responses during myocardial infarction (MI) are unknown. During the first 72 hours post-MI, neutrophils, M1 macrophages (pro-inflammatory), and M2 macrophages (pro-resolution) and the release of myeloperoxidase (MPO) and matrix metalloproteinases (MMP) play a role in the development of cardiac rupture which is an uncommon, but fatal complication. We hypothesize that in the acute stage of MI, Ac-SDKP decreases the incidence of cardiac rupture and mortality by preventing the infiltration of immune cells and by decreasing the activation of MPO and MMP.

Methods: MI was induced by the ligation of the left descending coronary artery in C57 mice. Vehicle or Ac-SDKP (1.6 mg/kg/d) was infused via osmotic minipump. Cardiac immune cell infiltration was assessed by flow cytometry, cardiac MPO and MMP activities were measured at 24-48 hrs post-MI. The incidence of cardiac rupture and mortality was determined at 7 days post-MI. Neutrophil migration was studied in vitro by chemotaxis transwell assay. Results: In infarcted mice, Ac-SDKP decreased the incidence of cardiac rupture from 51.0% (26 of 51 animals) to 27.3% (12/44; p=0.015) and mortality from 56.9% (29/51) to 31.8% (14/44; p=0.019). Ac-SDKP also reduced the cardiac infiltration by the M1 macrophages (veh: 1,495±236 vs Ac-SDKP: 765±69 cells/heart, p=0.027), without affecting M2 macrophages. Ac-SDKP did not affect neutrophil and MPO activity in vivo and neither affected neutrophil chemotaxis in vitro. However, Ac-SDKP prevented the activation of MMP-9 (veh: 3,686±508 vs Ac-SDKP: 1,696±512 optical density units, p=0.029) in infarcted hearts. Conclusion: Ac-SDKP prevents cardiac rupture and mortality in acute MI. These protective effects of Ac-SDKP are associated with a decrease in pro-inflammatory M1 macrophage infiltration and MMP-9 activation. Perspective: Cardiac rupture is an uncommon, but fatal complication of MI that could be prevented by the administration of Ac-SDKP or a peptidase resistant analog.


Funding: No

Funding Component:
Photoacoustic Imaging - Novel In Vivo Quantification of Placental Hypoxia in a Mouse Model of Hypertensive Pregnancy

Liliya M Yamaleyeva, Tiffaney Bledsoe, K. Bridget Brosnihan, Wake Forest Sch of Med, Winston-Salem, NC

Placental hypoxia/ischemia induces abnormal maternal and neonatal outcomes including preeclampsia and intrauterine growth restriction (IUGR). The ability to accurately determine placental oxygenation in a non-invasive way and in real-time is highly important during pregnancy as it may allow for the early diagnosis of IUGR and preeclampsia. Photoacoustic imaging (PA) is a novel preclinical and emerging clinical tool that combines optical contrast of photoacoustic laser technology with high spatial resolution of ultrasound. PA measures tissue oxygen saturation (sO2) that reflects differences in absorption spectra for oxygenated and deoxygenated hemoglobin. By using photoacoustic features of VEVO LAZR high resolution ultrasound system (VisualSonics) in a three-dimensional mode we investigated the sensitivity and accuracy of PA for placental oxygenation in C57Bl/6 mice at day 14 of gestation. Furthermore, since nitric oxide deficiency is associated with upregulation of circulatory hypoxia markers, C57Bl/6 mice were chronically treated with the nitric oxide inhibitor, L-NAME via osmotic minipumps (50 mg/kg/day; days 13 to 18 of gestation). The comparisons between scanned vs. not scanned uteroplacental units showed that PA had no effect on fetal (scanned: 0.028±0.001 vs. not scanned: 0.03±0.001 g/maternal body weight; p>0.05) or placental (scanned: 0.002±0.003 vs. not scanned: 0.002±0.002 g/maternal body weight; p>0.05) weights in C57Bl/6 mice. Changing inhaled O2 from 100- to 20% resulted in average in 12.5% reduction in total placental sO2. Systolic blood pressures were higher in L-NAME-treated vs. C57Bl/6 mice (215.8±0.8 vs. sham 99.3±4.4 mmHg; p<0.05). L-NAME infusion decreased sO2 in all areas of the placenta: labyrinth (73.6±0.97 vs. 58.6±3.4%, p<0.05), mesometrial triangle (63.0±2.0 vs. 48.9±1.0%, p<0.05), and total placenta (68.2±1.7 vs. 54.4±2.2%, p<0.05). Placental labyrinth had higher sO2 vs. mesometrial triangle area in both L-NAME infused (58.6±3.4 vs. 48.9±1.0%, p<0.05) and in C57Bl/6 (73.6±0.97 vs. 63.0±2.0%, p<0.05) mice reflecting elaborate branching morphology of the labyrinth. Our data suggest that PA imaging can detect regional differences in placental sO2 non-invasively and at different physiological states.

L.M. Yamaleyeva: None. T. Bledsoe: None. K. Brosnihan: None.

Funding: Yes
Funding Component: National Center

Low-dose Aspirin During Pregnancy Does Not Prevent Augmentation of Arterial Contractions to Thromboxane A2 in a Rat Model of Pregnancy-Induced Hypertension

Oluwatobiloba Osikoya, Paresh A Jaini, An Nguyen, Melissa Valdes, Styliani Goulopoulou, Univ of North Texas Health Science Ctr, Fort Worth, TX

Background: Daily intake of low dose aspirin after 12 weeks of gestation is currently recommended as a preventative intervention in pregnancies at high risk of developing preeclampsia. We previously showed that treating pregnant rats with a synthetic ligand of Toll-like receptor 9 (TLR9; ODN2395) activated the innate immune system causing preeclampsia-like characteristics such as maternal hypertension, vascular oxidative...
stress, and augmented vascular responses to thromboxane A2 (TxA2). **Hypothesis:** Low-dose aspirin treatment would ameliorate ODN2395-induced augmented responses to TxA2 in maternal arteries. **Methods:** Pregnant rats were treated with ODN2395 (300 μg) or vehicle on gestational day (GD) 14, 16, 18. Daily low-dose aspirin treatment (1.5 mg/kgBW) started on GD10 and continued throughout gestation. Systolic blood pressure was measured using the tail-cuff method on GD19. On GD21, uterine (UTA) and mesenteric resistance artery (MES) responses to a TxA2 mimetic (U46619, 10⁻⁹–10⁻⁶M) was measured with a wire myograph. **Results:** Uterine arteries from the ODN2395+Aspirin-treated group had greater contractile responses to U46619 compared to all other groups [Emax, %KCl: Control (n=6): 109.2±8.8; ODN2395 (n=5): 124.4±20.2; Aspirin (n=5): 76.0±11.2; ODN2395+Aspirin (n=5): 134.7±4.5, p<0.05]. Aspirin alone reduced MES responses to U46619 compared to control and MES from ODN2395 and ODN2395+Aspirin groups had greater contractile responses to U46619 compared to Aspirin alone [Emax, %KCl: Control (n=8): 109.8±5.2; ODN2395 (n=6): 111.1±4.3; Aspirin (n=7): 89.4±3.6; ODN2395+Aspirin (n=8): 121.7±6.3, p<0.05]. Rats treated with ODN2395 and ODN+Aspirin had greater systolic blood pressure compared to control rats [control (n=8): 97.3±3.2 mmHg, ODN2395 (n=6): 121.3±4.3 mmHg, Aspirin (n=8): 100.3±2.9 mmHg, ODN2395+Aspirin (n=7): 127.5 ±6.7 mmHg, p<0.05]. **Conclusion:** Exposure to a TLR9 agonist during pregnancy resulted in augmented contractile responses to a TxA2 mimic in maternal arteries when rats were in a regime of low-dose aspirin. This was shown in arteries from both reproductive and non-reproductive vascular beds.

O. Osikoya: None. P.A. Jaini: None. A. Nguyen: None. M. Valdes: None. S. Goulopoulou: None. Funding: Yes

---

**Placental Ischemia Causes Mitochondrial Dysfunction in Reduced Uterine Perfusion Pressure Rats**

**Venkata Ramana Vaka,** Mark W Cunningham Jr, Tarek Ibrahim, Lorena M Amaral, Babbette LaMarca, Univ of Mississippi Med Ctr, Jackson, MS

**Introduction:** Placental ischemia is believed to be the initial event in the development of preeclampsia (PE). PE is characterized by new onset hypertension and is associated with reduced fetal weight and placental oxidative stress. Mitochondrial dysfunction is the major cause of reactive oxygen species (ROS) generation. We hypothesize that the placental ischemia causes mitochondrial dysfunction in the reduced uterine perfusion pressure (RUPP) rat model of PE. Thus, the purpose of this study is to examine the effect of placental ischemia on mitochondrial function. **Methods:** Female Sprague Dawley rats were dived into two groups; normal pregnant (NP) and RUPP rats. On gestational day (GD) 14, RUPP surgery was performed, GD18 carotid catheters were inserted, and GD19 conscious blood pressure (MAP) was measured. GD 19 placentas were collected and mitochondria were isolated for respiration measurements. Respiration measurements included: basal state (isolated mitochondria with no substrates), state 2 (glutamate and malate as complex I substrates), state 3 (ADP stimulated), leak state (oligomycin induced ATP synthase inhibition), and maximal state (FCCP stimulated uncoupled). Cytochrome C oxidase (complex IV) activity was also measured in isolated mitochondria. **Results:** MAP was elevated in RUPP (n=16) compared to NP rats (n=14) (119±2 vs. 100±2 mmHg, p<0.05). State 3 (149±8 vs 220±12 mmHg, p<0.05).

**Funding Component:** National Center 031
pmol/sec/mg, p<0.05) and maximal (123±8 vs 159±5 pmol/sec/mg, p<0.05) respiration rates were significantly reduced in RUPP (n=5) vs NP (n=7) rats. However, basal, state 2, and state 4 respiratory rates were not different between RUPP vs NP. Respiratory control ratio (state 3/ state 4) was significantly reduced in RUPP (n=5) vs NP (n=7) (7±1 vs 10±1, p<0.05). Complex IV activity was reduced in RUPP (n=5) vs NP (n=6) rats (508±14 vs 644±17 nmol e-/min/mg, P<0.05). Conclusion: Reduced mitochondrial respiration and cytochrome c oxidase activity, indicators of mitochondrial dysfunction, occur in response to placental ischemia during pregnancy. Thus, suggesting that mitochondrial dysfunction may contribute to the pathophysiology of oxidative stress and hypertension during PE.

V. Vaka: None. M.W. Cunningham: None. T. Ibrahim: None. L.M. Amaral: None. B. LaMarca: None.

Funding: No

Funding Component:

032

Soluble fms-like Tyrosine Kinase-1 (sFlt-1)/Placental Growth Factor (PIGF) Imbalance Disrupts Vascular and Uteroplacental Gelatinolytic Matrix Metalloproteinase MMP-2 and -9 and Collagenolytic MMP-1 and -7 Balance in Hypertensive Pregnancy

Ning Cui, ZongLi Ren, Raouf A. Khalil, Harvard Medical Sch, Boston, MA

Preeclampsia is a complication of pregnancy manifested as hypertension-in-pregnancy (HTN-Preg) and fetal growth restriction. Placental ischemia could be an initiating event, but the linking mechanisms and tissue targets are unclear. Placental ischemia is associated with increased sFlt-1/PIGF ratio, and we have shown changes in MMPs in pregnant rat model of reduced uterine perfusion pressure (RUPP). To test the hypothesis that sFlt-1/PIGF imbalance could target vascular and uteroplacental MMPs, we tested if raising sFlt-1/PIGF ratio by infusing sFlt-1 (10 µg/kg/day) in Preg rats would increase BP and alter MMPs, and if correcting sFlt-1/PIGF ratio by infusing PlGF (20 µg/kg/day) in RUPP rats improves BP and reverses the changes in MMPs. On day 19, BP was recorded and the aorta, placenta, uterus and uterine artery were isolated for measuring MMP activity using gelatin and casein zymography and protein levels of MMPs and collagen I and IV using Western blots. BP was in Preg+sFlt 121±3 > Preg 93±7 and in RUPP+PlGF 97±4 < RUPP 118±4 mmHg. Litter size and pup weight were in Preg+sFlt < Preg and in RUPP+PlGF > RUPP. MMP-2 levels were in aorta of Preg+sFlt 3.30±0.05 < Preg 7.75±0.13, and in RUPP+PlGF 6.70±0.19 > RUPP 2.62±0.12. Similarly, MMP-2 in placenta, uterus and uterine artery and MMP-9 in all tissues were in Preg+sFlt < Preg and in RUPP+PlGF > RUPP. In contrast, MMP-1 levels were in aorta of Preg+sFlt 1.73±0.33 > Preg 0.55±0.49, and in RUPP+PlGF 0.53±0.18 < RUPP 4.04±0.30. Also, MMP-1 in placenta, uterus and uterine artery and MMP-7 in all tissues were in Preg+sFlt > Preg and in RUPP+PlGF < RUPP. The MMP-2 and -9 substrate collagen IV was in aorta of Preg+sFlt 0.16±0.00 > Preg 0.06±0.01, and in RUPP+PlGF 0.01±0.00 < RUPP 0.18±0.01. Similarly, collagen IV in placenta, uterus and uterine artery and the MMP-1 and -7 substrate collagen I in all tissues were in Preg+sFlt > Preg and in RUPP+PlGF < RUPP. Thus, sFlt-1/PIGF imbalance is a likely mechanism linking placental ischemia to decreased gelatinases, increased collagenases and improper vascular and uteroplacental remodeling. Correcting sFlt-1/PIGF balance by infusing PlGF could rectify the balance between gelatinolytic and collagenolytic MMPs and thereby restore proper vascular and uteroplacental remodeling in HTN-Preg.
The pathogenesis of preeclampsia (PreE) involves the failure of the maternal immune system to normally tolerate the pregnancy. Inflammatory cytokines are elevated in PreE-affected women with a concurrent decrease in anti-inflammatory cytokine production. Consistent with what other groups have observed in mouse models of hypertension during pregnancy and in human PreE-affected pregnancies, we observed increased inflammatory cytokine production and CD4+ T helper populations in our chronic infusion of vasopressin (AVP) mouse model of PreE. The mechanisms of immune modulation by AVP have not been elucidated. As increased T cell activity is involved in the development of PreE, the objective of this study was to investigate if CD4+ T cells express AVP receptors. Splenic CD4+ T cells were negatively purified from C57BL/6J saline and AVP-infused (24 ng/hour) dams. Expression of AVP receptors (AVPR) 1a, 1b, 2, and the aminopeptidase LNPEP (catalyzes AVP degradation) was determined via qPCR. Raw cycle threshold (Ct) values were normalized (∆Ct) against the 18S rRNA endogenous control. Mouse CD4+ T cells express all AVP receptors and LNPEP. By ANOVA, AVPR2 is the highest expressed receptor in CD4+ T cells from saline (N=7, p=0.002) and AVP-infused (N=10, p<0.0001) dams. Human maternal mononuclear cells, obtained from the University of Iowa Maternal-Fetal Tissue Bank (IRB #200910784) from control and PreE-affected women, were similarly analyzed. As in mouse CD4+ T cells, human control (N=27, p<0.0001) and PreE-affected (N=26, p<0.0001) CD4+ T cells most highly expressed AVPR2. AVPR1a was also highly expressed while AVPR1b was the least expressed. CD4+ T cells isolated from human PreE-affected women expressed significantly lower AVPR1a (10.0±0.3 N=27 vs. 11.1±0.2 N=0.23, p=0.009) and increased LNPEP (17.2±0.5 N=27 vs. 15.1±0.3 N=26, p=0.001) than controls. Here, we demonstrate CD4+ T cells, both mouse and human, express AVP receptors and that 1a and 2 are highest expressed. Although the actions of AVP on the vasculature are primarily mediated through AVPR1a, these data suggest AVP may differentially act through AVPR1a to mediate immune responses during PreE.
already received); Significant; NIH, AHA. J.L. Grobe: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectual property); Modest; Patent on Diagnostics and Therapeutics in Preeclampsia. M.K. Santillan: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectual property); Modest; Patent on Diagnostics and Therapeutics in Preeclampsia.

Funding: Yes
Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

Characterization of A Novel Maternally Restricted Pro-angiogenic Therapeutic for Preeclampsia

William H Stewart, Eric George, Gene L Bidwell III, Heather Chapman, Fakhri Mahdi, Logan Wilson, Univ of Mississippi Medical Ctr, Jackson, MS

Background: Preeclampsia is a major obstetrical health concern, affecting 5-8% of all pregnancies. Hallmarked by hypertension and endothelial dysfunction the origin of the disease remains obscure, though it is generally accepted that placental insufficiency/ischemia is a central cause. In response, the placenta secretes pathogenic factors, in particular the anti-angiogenic protein sFlt-1. Currently, there is no effective therapy for the management of the preeclampsia patient. We have recently produced a novel synthetic peptide based on placental growth factor (PIGF) which is maternally restricted by fusion to the synthetic carrier elastin like polypeptide (ELP). Here, we describe its in vivo pharmacokinetics and biodistribution.

Methods: Fluorescently labeled ELP-PLGF was administered i.v. and blood sampled serially to determine clearance kinetics. Long-term pharmacokinetics and biodistribution was performed after subcutaneous administration of labeled peptide. Measurements were made on serially drawn blood, and in the whole animal by in vivo imaging.

Results: ELP-PIGF exhibited markedly more favorable pharmacokinetics than the normal half life of PIGF, with a terminal half-life of \(~10\) hours as opposed to \(~30\) minutes for PIGF alone. Chronic administration found highest levels accumulating in placenta and kidney (two favorable targets for preeclampsia) and liver. A single subcutaneous administration at 100mg/kg resulted in sustained therapeutic plasma concentrations for over 10 days.

Conclusion: These data demonstrate that ELP-PIGF has favorable pharmacokinetic and biodistribution profiles. Previous data suggest ELP-PIGF directly antagonizes sFlt-1 in culture. Future studies to assess the in vivo effectiveness of ELP-PIGF in managing placental ischemia induced hypertension and endothelial dysfunction are currently in progress.

Acknowledgment: This work was supported by NIH grants R0121527 (GLB), T32HL105324 (OCL), P01HL51971, P20GM104357 (EMG), and R00HL116774 (EMG)

Differential Effects of Bardet-biedl Syndrome 1 Gene Deletion From Agouti-related Peptide Neurons

Deng F Guo, The Univ of Iowa, Iowa, IA; John J. Reho, Donald A. Morgan, Kamal Rahmouni, the Univ of Iowa, Iowa, IA

Bardet-Biedl syndrome (BBS) is a pleiotropic autosomal recessive human disorder associated with several clinical features including obesity and hypertension. We previously demonstrated that Bbs1 gene deletion from the central nervous system caused obesity and hypertension in mice highlighting the importance of neuronal BBS proteins for energy homeostasis and cardiovascular regulation. Here, we investigated the role of Bbs1 gene in the orexigenic agouti-related peptide (AgRP) neurons. We crossed Bbs1fl/fl mice with AgRPcre mice and confirmed Cre recombinase activity in AgRP neurons using td-Tomato reporter mice. Interestingly, female AgRPcre/Bbs1fl/fl mice developed profound obesity as indicated by the increased body weight (46.8±2.0 g vs control littersmates, 35.5±1.9 g at 25 weeks of age). In contrast, body weight of male AgRPcre/Bbs1fl/fl mice (48.5±1.2 g) was similar to the controls (46.7±1.2 g). There was no change in the expression of hypothalamic genes regulating food intake (AgRP, proopiomelanocortin and neuropeptide Y) in AgRPcre/Bbs1fl/fl mice relative to controls. Next, we assessed the consequence on arterial pressure (AP) and sympathetic nerve activity (SNA) of ablating the Bbs1 gene from AgRP neurons. Interestingly, deletion of the Bbs1 gene in AgRP neurons did not recapitulate the hypertension phenotype of BBS as indicated by the lack of difference in mean AP (107±5.0 vs 106±5.2 mmHg in controls) and heart rate (597±23.5 vs 589±16.4 in control). However, conscious renal SNA was significantly higher in AgRPcre/Bbs1fl/fl mice relative to controls (114±10 vs 78±6 spikes/sec, p<0.05). In addition, the depressor effect of ganglionic blockade (hexamethonium) was higher in AgRPcre/Bbs1fl/fl mice (-64.4±10 vs -55.8±8.3 mmHg in control). Finally, we used wire myography to measure mesenteric artery vascular function and found impairment in acetylcholine induced relaxation in AgRPcre/Bbs1fl/fl mice (Max. relaxation: 78 ± 6% vs 26 ± 9% in controls; p<0.05), but no changes in the relaxation evoked by sodium nitroprusside, suggesting endothelial, but not smooth muscle, dysfunction. These findings demonstrate that Bbs1 gene in AgRP neurons is critical for energy homeostasis, renal sympathetic outflow and vascular endothelial function.


Mitoprotection Preserves the Cytoskeleton and Alleviates Myocardial Injury in Early Swine Metabolic Syndrome

Fang Yuan, Henan Provincial People's Hosp, Zhengzhou, China; John R Woollard, Kyra L Jordan, Amir Lerman, Lilach O Lerman, Alfonso Eirin, Mayo Clinic, Rochester, MN

Background: The mechanisms responsible for cardiac injury in the early stage of metabolic syndrome (MetS) remain unknown. Mitochondria are intimately associated with myofibrils, where the cytoskeleton functions as
a linkage coordinator. We hypothesized that early MetS is characterized by cytoskeletal-mitochondrial disorganization, and that mitoprotection with mitochondria-targeted peptides (MTP) would preserve cytoskeletal-mitochondrial structure and attenuate myocardial injury in early swine MetS. Methods: Pigs were studied after 16wks of diet-induced MetS, MetS treated for the last 4wks with the MTP Elamipretide (0.1mg/kg SC q.d), and Lean controls (n=6 each). Cardiac function (multidetector CT) was assessed in-vivo, and mitochondrial structure (electron microscopy), the mitochondrial inner membrane phospholipid cardiolipin (mass spectrometry), cytoskeletal proteins, oxidative stress, and apoptosis ex-vivo. Results: MetS pigs developed hypertension and insulin resistance, yet cardiac function was preserved. MetS induced loss of desmin and tubulin that was paralleled by mitochondrial disorganization, decreased 18:2 cardiolipin, and increased oxidative stress and apoptosis. MTP slightly improved cardiolipin species profile, restored mitochondrial organization, and upregulated cytoskeletal proteins, attenuating apoptosis and oxidative stress. Conclusion: Early MetS leads to disorganization of the cardiomyocyte cytoskeletal- mitochondrial architecture and cardiac injury. MTP may provide a novel therapeutic potential for improving cardiac injury in early MetS, and potentially preventing future deterioration in cardiac function.
Obese subjects are often resistant to leptin’s metabolic effects although blood pressure (BP) and sympathetic nervous system responses appear to be preserved. Protein tyrosine phosphatase 1B (PTP1B), a negative regulator of leptin signaling, may play a role in promoting this selective leptin resistance and causing metabolic dysfunction in obesity. Our previous studies suggest that the chronic BP responses to leptin are mediated via activation of pro-opiomelanocortin (POMC) neurons. The goal of this study was to determine if PTP1B in POMC neurons differentially controls metabolic functions and BP in mice fed a high fat diet (HFD). Male mice with POMC specific PTP1B deletion (POMC/PTP1B−/−) and littermate controls (PTP1Bflox/flox) were fed a HFD from 6 to 22 wks of age. Baseline BP after 16 weeks of a HFD (95±2 vs. 95±3 mmHg) and BP responses to acute stress (Δ32±0 vs. Δ32±6 mmHg), measured by telemetry, were not different in POMC/PTP1B−/− compared to control mice, respectively. Heart rate (HR) was not different in POMC/PTP1B−/− and control mice during acute stress (699±4 vs. 697±15 bpm, respectively). Total body weight (TBW) and fat mass were reduced at 20 weeks of age in POMC/PTP1B−/− compared to controls (36.7±0.1 vs. 42.0±1 g TBW and 12.7±0.4 vs. 16.1±1.0 g fat mass, respectively). Liver weight of POMC/PTP1B−/− mice was less than in controls, and this was evident even when liver weight was normalized as % of TBW (4.5±0.2 vs. 5.0±0.2 %). POMC/PTP1B−/− males had reduced liver lipid accumulation compared to controls as measured by EchoMRI (0.08±0.03 vs. 0.15±0.03 g/g liver weight). Glucose tolerance was also improved by 46% in POMC/PTP1B−/− compared to controls as measured by AUC, 25856±1683 vs. 47267±5616 mg/dLx120min, respectively. These findings indicate that PTP1B signaling in POMC neurons plays a crucial role in regulating liver lipid accumulation and glucose tolerance but does not appear to mediate changes in BP or BP responses to acute stress in mice fed a high HFD (supported by NHLBI-PO1HL51971 and NIGMS P20GM104357)


Funding: No

Funding Component:

038

Hyperglycemia Mediated Cardiac Damage in High-Fat Fed E487K Aldehyde Dehydrogenase 2 (ALDH 2*2) Mutant Diabetic Mice: Role for ALDH2 Activation

Guodong Pan, Suresh Palaniyandi, Henry Ford Health System, Detroit, MI

Aldehyde dehydrogenase 2 (ALDH2), a mitochondrial enzyme in the heart, detoxifies reactive aldehydes and protects heart from oxidative stress. East Asians (~700 million) are carriers of E487K point mutation of ALDH2 (ALDH2*2) with intrinsically low ALDH2 activity. ALDH2*2 is associated with increased maternal inheritance of diabetes mellitus (DM), and DM-induced neuropathy and vasculopathy. However the pathophysiology of diabetic cardiac damage is not studied in ALDH2*2 carriers. DM is a polygenic disease and DM-induced cardiac damage may be multifactorial. However we hypothesis that hyperglycemia-induced oxidative stress mediated 4-hydroxy-2-nonenal (4HNE) toxicity contributes to the cardiac damage in ALDH2*2 mutant mice (low intrinsic ALDH2 activity) with type-2 diabetes. We induced type-2 diabetes by feeding high-fat diet
and found they developed hyperglycemia (blood glucose (BG) levels increased to 357 ± 100 mg/dl vs 137 ± 7 mg/dl) and insulin resistance as measured by glucose tolerance test (GTT) (BG levels 408 ± 50 mg/dl vs 165 ± 18 mg/dl at 2 hours after GTT). To delineate the role of hyperglycemia, we treated the diabetic mice with a sodium-glucose co-transporter 2 (SGLT2) inhibitor, Empaglifuzin (EMP) (3mg/kg/day) or Vehicle for 8 weeks. EMP reduced BG levels from 502 ± 75 mg/dl to 193 ± 50 mg/dl by enhancing urinary glucose excretion. Surprisingly EMP reversed insulin resistance as maintained similar BG levels before and after 2 hours of GTT; 190 ± 23 mg/dl vs 188 ± 16 mg/dl. EMP also increased ALDH2 activity to 22 ± 8 % from 7 ± 3 % and 4HNE protein adduct levels. Finally EMP improved cardiac function i.e. % fractional shortening (FS) is increased to 70 ± 4 compared to 53 ± 10. Our data suggested hyperglycemia partially contribute to the diabetic cardiac damage via increasing 4HNE protein adducts. Alda-1 (10 mg/kg/day) treatment further augmented ALDH2 activity, reduced 4HNE adducts and improved cardiac function in EMP-treated ALDH2*2 mice. Thus hyperglycemia mediated secondary events in type-2 DM are significant pathomechanism of the cardiac damage. In conclusion, we propose ALDH2 activation may ameliorate diabetic patients from cardiac complications who receive glucose lowering treatments.

G. Pan: None. S. Palaniyandi: None.

Funding: Yes
Funding Component: National Center 039

Frederique B Yiannikouris, Dept of Pharmacology and Nutritional Sciences, Lexington, KY

Previous findings from our laboratory have demonstrated that adipocyte (pro)renin receptor deficiency induced profound metabolic disturbances leading to lipodystrophy, liver steatosis and increased in blood pressure. The purpose of the study was to determine whether adipocyte (pro)renin receptor (PRR) deficiency leads to similar physiological consequences when the deletion of adipocyte PRR is induced at adulthood. Male mice expressing an inducible adipocyte-specific Cre under the control of the adiponectin promotor (C57BL/6-Tg (Adipoq-cre/ERT2)1Soff/J, Jackson Laboratory, USA) were bred to female PRR<sup>f/Y</sup> mice (n=4-5 mice/group). Control (PRR<sup>f/Y</sup>) and inducible adipocyte-PRR-deficient mice (PRR<sup>adi-CreERT<sup>y</sup></sub>) were fed a standard diet. At 8 weeks of age, control and PRR<sup>adi-CreERT<sup>y</sup></sub> mice were injected intraperitonally on 5 consecutive days with tamoxifen (40 mg/kg of tamoxifen in sunflower oil). The expression of PRR gene was significantly decreased in adipose tissue of PRR<sup>adi-CreERT<sup>y</sup></sub> mice compared with control mice (PRR<sup>f/Y</sup>: 29±3g; PRR<sup>adi-CreERT<sup>y</sup></sub>: 27±1g). White adipose tissue and fat masses were significantly reduced in PRR<sup>adi-CreERT<sup>y</sup></sub> mice compared with control mice (PRR<sup>f/Y</sup>: 0.97±0.12g; PRR<sup>adi-CreERT<sup>y</sup></sub>: 0.24±0.07g). Liver weights were not significantly different between PRR<sup>adi-CreERT<sup>y</sup></sub> and control mice (Liver: PRR<sup>f/Y</sup>: 1.6±0.2g; PRR<sup>adi-CreERT<sup>y</sup></sub>: 1.7±0.1g) suggesting the absence of hepatic steatosis. Gene expression analysis in white adipose tissues revealed a

Adipocyte (pro)renin-receptor is a Master Regulator of Adipocyte Differentiation

Frederique B Yiannikouris, Dept of Pharmacology and Nutritional Sciences, Lexington, KY
downregulation of genes involved in adipocyte differentiation (CREB, PPARγ, CEBPα and CEBPβ) and lipid synthesis and trafficking (FAS and CD36) confirming the specificity of PRR to regulate adipocyte differentiation in white adipose tissue. Our data suggest that PRR is a master regulator of adipocyte differentiation. The expression of gene of the renin angiotensin system and blood pressure are currently under investigation.

F.B. Yiannikouris: None.

Funding: Yes
Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

040

Comparison of Effects of Reux en Y Gastric Bypass and Intra-abdominal Lipectomy on Cardiovascular Function in the Metabolic Syndrome

Amanda Soler, Brenda Hutcheson, Jenny Yang, New York Medical Coll, Valhalla, NY; Chastity Bradford, Tuskegee Univ, Tuskegee, AL; Frank Zhang, Katie Gotlinger, Michal L. Schwartzman, Petra Rocic, New York Medical Coll, Valhalla, NY

Central (visceral) obesity is a key feature of the metabolic syndrome and an independent predictor of cardiovascular disease. Reux en Y gastric bypass (RnY) has been shown to offer protection against cardiovascular disease, but residual risk remains. It is also unknown whether the cardiovascular benefit is a consequence of a decrease in visceral (intra-abdominal) adipose tissue or of other factors. In this study, we compared the effects of RnY vs. removal of 90% of visceral adipose tissue (=5% body weight) by intra-abdominal lipectomy on cardiac function (echocardiography), macrovascular function (carotid artery stiffness) and microvascular function (coronary artery endothelium-dependent vasorelaxation) in a metabolic syndrome rat model (JCR:LA-cp, JCR).

Cardiac output (CO) and ejection fraction (EF) were significantly decreased in JCR vs. normal (Sprague-Dawley, SD) rats (CO=50±5%, EF=45±2% of normal), and were significantly improved by both RnY and intra-abdominal lipectomy (CO=75±6%, EF=82±2% and CO=80±3%, EF=90±2% of normal, respectively). Likewise, acetylcholine-dependent coronary artery vasorelaxation was impaired in JCR rats (50±1% of normal), and was significantly improved by both RnY and intra-abdominal lipectomy (98±2% and 98±3% of normal, respectively). Carotid artery stiffness was significantly increased in JCR rats (~2 fold vs. SD), and was normalized by intra-abdominal lipectomy (to equal SD), but not by RnY (~2 fold vs. SD). Intra-abdominal lipectomy but not RnY also decreased cardiac and vascular elastin degradation in JCR rats (Lipectomy: ~50% (heart), ~75% (carotid); RnY: ~15% (heart), ~5% (carotid) vs. untreated JCR, respectively), concomitant with a decrease in matrix metalloproteinase 12 (MMP12), a major elastase, activation (~50% (heart), ~75% (carotid), ~87% (visceral fat), ~75% (circulating) vs. untreated JCR) and in 20-hydroxyeicosatetraeonic acid (20-HETE) levels (~4 (heart), ~7 (carotid), ~4 (visceral fat), ~4 (circulating) fold vs. untreated JCR). Thus, our data indicate that intra-abdominal adipose tissue itself is a source of factors that may be important negative regulators of micro- and macrovascular and cardiac function, but are not eliminated by RnY.


Funding: No
Funding Component:
mTORC1 is Required for Leptin-induced Sympathetic Activation to the Kidney but Not Brown Adipose Tissue

Balyssa B Bell, Donald A Morgan, Kamal Rahmouni, Univ of Iowa, Iowa City, IA

The adipocyte-derived hormone leptin plays a critical role in the regulation of energy homeostasis through its action in the brain to decrease food intake and promote energy expenditure by increasing sympathetic nerve activity (SNA) to the thermogenic brown adipose tissue (BAT). Leptin also increases SNA to cardiovascular organs including the kidney and raises arterial pressure. However, it is unclear whether leptin controls regional SNA via conserved or distinct molecular mechanisms. Multiple intracellular pathways have been associated with leptin signaling including the mechanistic target of rapamycin complex 1 (mTORC1), which has been proposed as a critical determinant of leptin action. Here, we assessed the contribution of mTORC1 signaling to leptin-evoked regional sympathetic activation. Simultaneous multifiber recording of renal and BAT SNA in anesthetized C57BL/6J mice showed that intracerebroventricular (ICV) administration of leptin (2µg, n=5) increased both renal (170±34%) and BAT (208±37%) SNA. Interestingly, ICV pre-treatment with the mTORC1 inhibitor (rapamycin, 5ng, n=6) abolished the leptin-induced increase in renal (10±6%, P<0.05 vs controls) but not BAT (226±31%) SNA. Next, we used conditional knockout mice that lack the critical mTORC1 subunit, Raptor, specifically in leptin receptor (LRb)-expressing cells (LRbCre/Raptorfl/fl) to determine the long-term effects of disrupting mTORC1 signaling on leptin-evoked increase in regional SNA. We confirmed the inability of leptin to activate mTORC1 signaling in LRb-expressing cells of LRbCre/Raptorfl/fl mice relative to controls using immunohistochemical staining of phosphorylated ribosomal S6, a downstream target of mTORC1. We observed a significant increase in renal SNA in response to ICV leptin in control mice (127±16%, n=9), but not in LRbCre/Raptorfl/fl mice (-4±15%, n=9, P<0.05 vs controls). Conversely, ICV leptin-induced increase in BAT SNA was not different in LRbCre/Raptorfl/fl mice (109±27%, n=5) vs. littermate controls (173±52%, n=4). Our data suggest a critical role for mTORC1 signaling in selectively mediating the cardiovascular sympathetic but not the thermogenic actions of leptin, with important implications for obesity-associated hypertension.

B.B. Bell: None. D.A. Morgan: None. K. Rahmouni: None.

Funding: Yes
Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

Selective Maternal Hepatic Silencing of Angiotensinogen in Reduced Uterine Placental Perfusion Pressure Rats Improves Blood Pressure and Fetal Weights

Mark W Cunningham Jr., Univ. Of Mississippi Medical Ctr, Jackson, MS; Nadine Haase, Experimental and Clinical Res Ctr Helios Clinic, Berlin, Germany; Jessica L Faulkner, Tarek Ibrahim, Venkata Ramana Vaka, Univ. Of Mississippi Medical Ctr, Jackson, MS; Ralf Dechend, Experimental and Clinical Res Ctr Helios Clinic, Berlin, Germany; Don Foster, Alnylam Pharmaceuticals, Cambridge, MA; Babbette LaMarca, Univ. Of Mississippi Medical Ctr, Jackson, MS

Women with Preeclampsia (PE), a form of new onset hypertension during pregnancy, exhibit
alterations in the renin angiotension system (RAS). These differences include angiotensin II type 1 receptor agonistic autoantibodies (AT1-AA), ANG II sensitivity, and increased oxidation and levels of angiotensinogen (AGT). While antihypertensive drugs are used in pregnancy, their use is associated with reduced fetal growth, and RAS blockade is specifically contraindicated due to fetotoxicity. AGT is the precursor to ANG II and the initial substrate of the RAS pathway. Genetic mutations that result in an over-expression of AGT are strongly associated with hypertension. We have previously shown that siRNA targeting maternal hepatic AGT in a transgenic model of preeclampsia (hAGT x hREN) resulted in lower blood pressure, a 90% reduction in AT1-AA activity, and normalized IUGR. The purpose of the present study is to examine the effect of silencing maternal hepatic AGT on maternal blood pressure and fetal growth in the reduced uterine placental perfusion pressure (RUPP) model of PE. **Methods:** Pregnant Sprague Dawley rats were divided into 4 groups: normal pregnant (NP, n=7); RUPP (n= 6); RUPP + siRNA (n =7); NP + siRNA (n=7). On day 12 of gestation (GD), siRNA was subcutaneously injected into rats (10mg/kg), GD14 the RUPP surgery was performed, GD18 carotid catheters inserted, and GD19 conscious blood pressure (MAP) and fetal weight was recorded. **Results:** MAP was elevated in RUPP vs. NP (134±6 vs. 101±4 mmHg, p< 0.05), but was lower in RUPP + siRNA (117±4 mmHg) and unchanged in the NP+siRNA (92±3 mmHg) group compared to NP. Average fetal weights by group were 2.30±0.09 g (NP), 1.89±0.10 g (RUPP), 2.38±0.09 g (NP+siRNA), and 2.12±0.06 (RUPP+siRNA). A one-way ANOVA indicates a significant reduction in pup weights between NP and RUPP, but not NP+siRNA and RUPP+siRNA, indicating administration of siRNA to RUPP rats prevented the decrease in fetal weight. **Conclusion:** Administration of AGT siRNA to RUPP rats blunts the increase in blood pressure and prevents a decrease in fetal weights in response to placental ischemia and thus could be a potential therapy for management of preeclampsia and other hypertensive disorders of pregnancy. **Research Supported by Alnylam & T32HL105324,**

M.W. Cunningham: None. N. Haase: None. J.L. Faulkner: None. T. Ibrahim: None. V. Vaka: None. R. Dechend: None. D. Foster: None. B. LaMarca: None.

Funding: No
Funding Component:

043

**Dendritic Cells Mediate Renal T Cell Activation in Hypertension**

Nathan P Rudemiller, Jiandong Zhang, Gianna E Hammer, Yen-Rei A Yu, Robert Griffiths, Michael D Gunn, Steven D Crowley, Duke Univ, Durham, NC

Activated T lymphocytes exacerbate hypertension in part by infiltrating the kidney to promote sodium retention. Dendritic cells (DCs) are the most potent antigen presenting cells that activate T cells and have been shown to contribute to hypertension. The current studies therefore explored whether DC-mediated activation of renal T cells can exaggerate the chronic hypertensive response to angiotensin (Ang) II. First, we confirmed that renal T cells undergo DC-mediated activation during the initiation of hypertension by analyzing immune cell populations in renal tissue via flow cytometry. In Fms-like tyrosine kinase 3 ligand-deficient (FLT3L KO) mice that lack DCs, the proportions of effector (CD44hiCD62lo) T cells in the kidney were similar to wild-type (WT) controls at baseline (50±3 vs. 52±7% of CD3+ cells). However, after 5 days of Ang II-induced hypertension, the proportions of effector T cells...
were dramatically higher in the WT kidney versus the FLT3L KOs (69±3 vs. 52±3% of CD3^+ cells; p<0.01), indicating that DCs activate T cells in hypertension. As DCs activate T cells in local lymph nodes, we phenotyped T cell subsets in the kidney lymph node (KLN) following 4 weeks of hypertension and detected elevated proportions of effector CD4^+ T cells compared to baseline (10.7±2.0 vs. 6.9±0.8% of CD4^+ T cells). The ubiquitin-editing protein A20 in DCs suppresses their capacity to stimulate T cells. Thus, mice with heterozygous deletion of A20 in DCs (CD11c-cre A20^<sup>fl/wt</sup> = DC ACT) harbor spontaneously active DCs that enhance T cell activation. To test the contribution of DC-mediated T cell activation to hypertension, we measured blood pressures in WT and DC ACT mice during 4 weeks of chronic Ang II infusion (300ng/kg/min). While MAPs were similar in the 2 groups at baseline, the DC ACT mice had an exaggerated chronic hypertensive response (143±2 vs. 131±4 mmHg; p=0.04) with more severe cardiac hypertrophy (7.3±0.3 vs. 6.4±0.4 mg/gm body wt; p<0.04). The KLN from the DC ACT animals also contained higher proportions of effector T cells than controls (12.1±0.2 vs. 8.2±0.5% of CD3^+ cells; p<0.01). In conclusion, DC-mediated activation of T cells promotes blood pressure elevation by facilitating the accumulation of effector T cells in the kidney during hypertension.


Funding: Yes
Funding Component: Mid-Atlantic Affiliate (Maryland, North Carolina, South Carolina, Virginia & Washington, DC)

Deletion of Serum and Glucocorticoid-regulated Kinase 1 (SGK1) in T Cells Attenuates Hypertension and Renal/Vascular Dysfunction

Allison E Norlander, Vanderbilt Univ, Nashville, TN; Mohamed A Saleh, Mansoura Univ, Mansoura, Egypt; Arvind K Pandey, Hana A Itani, Vanderbilt Univ, Nashville, TN; Jing Wu, Iowa Univ, Iowa City, IA; Liang Xiao, Bethany L Dale, David G Harrison, Meena S Madhur, Vanderbilt Univ, Nashville, TN

We have previously shown that the T cell-derived pro-inflammatory cytokine, interleukin 17A (IL17A), is upregulated by and promotes angiotensin II (Ang II)-induced hypertension and contributes to renal and vascular dysfunction. It was recently demonstrated that an excess of 40 mM of sodium chloride enhances IL17A production from CD4+ T cells in an SGK1 dependent manner. Since dietary salt intake is associated with hypertension, we hypothesized that T cell SGK1 promotes hypertension and contributes to end-organ dysfunction. To test this hypothesis, we crossed SGK1<sup>fl/fl</sup> mice with Tg<sup>CD4cre</sup> mice to delete SGK1 in T lymphocytes. Loss of T cell SGK1 resulted in a blunted blood pressure response following 2 weeks of Ang II infusion (24.8 mmHg reduction, p=0.01). Moreover, renal and vascular inflammation in response to Ang II infusion was abrogated in these mice compared to SGK1<sup>fl/fl</sup> control mice. Ang II infusion increased total (CD45+) leukocytes in the kidney from 55.4 to 120.4 x10^3 (p<0.01) in SGK1<sup>fl/fl</sup> mice while there was no increase in mice with T cell deletion of SGK1 (48.1 to 47.5x10^3, p=ns). Similarly, Ang II increased total (CD45+) leukocytes in the aorta from 5.7 to 52.4x10^3 (p<0.01) in SGK1<sup>fl/fl</sup> mice compared to no increase in mice with T cell deletion of SGK1 (16.1 to 10.1x10^3, p=ns). Furthermore, relaxation of mesenteric arterioles isolated from Ang II infused SGK1<sup>fl/fl</sup> mice in response to acetylcholine was impaired by 22.37% (p<0.0001) compared to a 2.62% (p=ns) impairment in vessels isolated from mice with T cell deletion of SGK1, demonstrating that the latter group has preserved endothelial
function despite Ang II infusion. To assess renal dysfunction, we measured urinary albumin:creatinine ratio which increased 5.81-fold (p<0.01) in SGK1fl/fl mice infused with Ang II compared to 3.23-fold (p=ns) in mice without T cell SGK1, demonstrating that these mice are protected from Ang II-induced renal injury. Finally, we found that total numbers of splenic CD4+IL17A+ cells increase from 0.44% to 1.15% (p<0.05) in SGK1fl/fl mice infused with Ang II compared to no change (0.45% to 0.47%, p=ns) in mice without T cell SGK1. These studies demonstrate that T cell SGK1 may be a novel therapeutic target for hypertension and the associated end-organ dysfunction.


Funding: Yes
Funding Component: National Center
045

Endothelial Colony Forming Cells Dysfunction Relates to Cardiovascular Alterations in Preterm Born Adults

Mariane Bertagnolli, Katryn Paquette, Megan Sutherland, Marie-Amelie Lukaszewski, Ying He, Anik Cloutier, Rong Wu, Jean-Luc Bigras, Sainte-Justine Univ Hosp Res Ctr, Univ of Montreal, Montréal, QC, Canada; Bernard Thebault, Ottawa Hosp Res Inst, Ottawa, ON, Canada; Thuy Mai Luu, Anne Monique Nuyt, Sainte-Justine Univ Hosp Res Ctr, Univ of Montreal, Montréal, QC, Canada

Endothelial colony-forming cell (ECFC), a subtype of endothelial progenitor cells with high clonogenic and proliferative capacity, is present in cord and peripheral blood, participating in neovessel formation and regeneration. Cord blood ECFCs have impaired bioactivity in pregnancy complications and preterm (PT) birth. Dysfunction of cord blood ECFC is also related to complications of prematurity such as bronchopulmonary dysplasia. Although cardiovascular alterations, such as high blood pressure (BP) and left ventricular (LV) dysfunction, occur in PT subjects during adulthood, whether ECFC dysfunction beyond the neonatal period relates to such alterations is not known. OBJECTIVE: We aim in this study to assess if ECFC function relates to cardiovascular alterations in PT born adults. METHODS: Peripheral blood mononuclear cells from 30 young adults (21-28 years old) born very PT (<29 weeks gestation) and 30 at term (T, ≥37 weeks gestation) were separated by density gradient and cultured to ECFC colony formation. ECFC proliferative and angiogenic function were assessed in vitro by modified thymidine analogue (EdU) incorporation and tube formation in Matrigel. BP was measured by 24h monitor and LV mass index (g/cm²) by ultrasound imaging. All analyses were performed blind; correlations were significant when p<0.05. RESULTS: The proportion of early (<15 days) and late (≥15 days) time for ECFC colony formation was different between PT and T, with a higher frequency for late growth or no colony in the PT born group. Time to colony formation inversely correlated with ECFC proliferation rate (r=-0.57, r²=0.32) and tube formation (r=-0.57, r²=0.33) in PT, which shows ECFC dysfunction in those PT subjects with late colony formation, with no significant correlations in T. Additionally, time to colony formation has positively related to systolic BP (r=0.54, r²=0.29) and LV mass index (r=0.49, r²=0.24) in PT born subjects. CONCLUSION: Our findings demonstrate, for the first time, an association between dysfunctional circulating ECFCs and cardiovascular alterations in adults born PT. We also show that ECFC dysfunction, in PT adults, significantly relates to important cardiovascular
risk factors such as high BP and increased LV mass.


Funding: No

Funding Component:

The Role of Tumor Necrosis Factor α and Natural Killer Cells in Uterine Artery Function During Pregnancy in the Stroke Prone Spontaneously Hypertensive Rat

Heather Y Small, Ryszard Nosalski, Hannah Morgan, Elisabeth Beattie, Tomasz Guzik, Delyth Graham, Christian Delles, Univ of Glasgow, Glasgow, United Kingdom

Objective: We have previously characterised the stroke prone spontaneously hypertensive rat (SHRSP) as a model of deficient uterine artery remodelling and identified an increase in pro-inflammatory TNFα relative to the normotensive WKY strain during pregnancy.

Method: SHRSP were treated with etanercept (0.8 mg/kg) or vehicle at gestational day (GD) 0, 6, 12 and 18. Animals were sacrificed at GD18.

Results: Etanercept reduced systolic blood pressure in the SHRSP after GD12 (ΔSBP GD 10-21 SHRSP 12.0 ± 4.17 vs. ETN 25.8 ± 4.27 mmHg; p<0.05). Uterine arteries from GD18 showed that etanercept reduced uterine artery contraction (SHRSP 57.3 ± 8.75 vs. ETN 35.2 ± 2.19 kPa; p<0.01) and increased carbachol response (SHRSP 13.8 ± 3.8 vs. ETN 40.1 ± 3.25 %; p<0.05). Uteroplacental blood flow analysed using Doppler showed that etanercept reduced uterine artery resistance index in SHRSP (SHRSP 0.79 ± 0.02 vs. ETN 0.61 ± 0.02 UARI; p<0.01).

Etanercept increased litter size (SHRSP 7.80 ± 0.44 vs. ETN 12.75 ± 0.94 fetuses), reduced resorption frequency (SHRSP 66.7% vs. ETN 25.0% dams with resorption) and decreased glycogen cell loss from the placenta in SHRSP. We sought to identify the source of excess TNFα in the SHRSP. Natural killer (NK) cells (CD3-CD161+) were increased in the SHRSP relative to the WKY in the maternal circulation (WKY 1.5 ± 0.4 vs. SHRSP 6.06 ± 0.28 %; p<0.01) and placenta (WKY 11.6 ± 2.39 vs. SHRSP 659.8 ± 201.2 cells/mg; p<0.01). These NK cells produced excess TNFα in the SHRSP maternal circulation (SHRSP 6.5 ± 0.4 vs. WKY 2.5 ± 0.4 %; p<0.05) and placenta (SHRSP 65.7 ± 4.2 vs. WKY 16.9 ± 1.7 %; p<0.01) relative to the WKY. In the SHRSP placenta, etanercept treatment reduced the number of cytotoxic NK cells (SHRSP 659.8 ± 201.2 vs. ETN 148.0 ± 12.62 cells/mg; p<0.01) by down-regulating CD161 expression associated with a decrease in granzyme B production (CD161+ 71.47 ± 2.1 vs. CD161Low 14.32 ± 0.77 % granzyme B+; p<0.01).

Conclusions: Excess TNFα plays a causative role in adverse pregnancy outcome in the SHRSP. One source of this TNFα is an increase in NK cells during gestation in the SHRSP. Etanercept targets NK cells in the SHRSP placenta and down-regulates cytotoxic granzyme B production.


Funding: No

Funding Component:

Activation of Sirt1 Deacetylase Attenuates Klotho Deficiency-induced Arterial Stiffness and Hypertension
Objective. Arterial stiffness is an independent risk factor for stroke and myocardial infarction. This study was designed to investigate the role of SIRT1, an important deacetylase, and its relationship with Klotho, a kidney-derived aging-suppressor protein, in the pathogenesis of arterial stiffness and hypertension.

Methods and Results. We found that the serum level of Klotho was decreased by nearly 45% in patients with arterial stiffness and hypertension. Interestingly, Klotho haplodeficiency caused arterial stiffening and hypertension, as evidenced by significant increases in pulse wave velocity (PWV) and blood pressure (BP) in Klotho-haplodeficient (KL+/−) mice. Notably, the expression and activity of SIRT1 were decreased significantly in aortic endothelial and smooth muscle cells in KL+/− mice, suggesting that Klotho deficiency downregulates SIRT1. Treatment with SRT1720 (15 mg/kg/day, IP), a specific SIRT1 activator, abolished Klotho deficiency-induced arterial stiffness and hypertension in KL+/− mice. Klotho deficiency was associated with significant decreases in activities of AMP-activated protein kinase alpha (AMPKα) and endothelial nitric oxide synthase (eNOS) in aortas, which were abolished by SRT1720. Furthermore, Klotho deficiency upregulated NADPH oxidase activity and superoxide production, increased collagen expression, and enhanced elastin fragmentation in the media of aortas. These Klotho deficiency-associated changes were blocked by SRT1720.

Conclusion. This study provides the first evidence that Klotho deficiency downregulates SIRT1 activity in arterial endothelial and smooth muscle cells. Pharmacological activation of SIRT1 is likely to be an effective therapeutic approach for arterial stiffness and hypertension.

Expression of a Hypertension-causing Mutation in Cullin 3 (CUL3Δ9) Specifically in Smooth Muscle Causes Vascular Dysfunction and Hypertension

Larry N. Agbor, Jing Wu, Stella-Rita C. Ibeawuchi, Chunyan Hu, Deborah R. Davis, Henry L. Keen, Frederick W. Quelle, Curt D Sigmund, Univ of Iowa Carver Coll of Med, Iowa City, IA

Pseudohypoaldosteronism type II (PHAII) patients with mutations in cullin 3 (CUL3) resulting in exon 9 deletion (CUL3Δ9), exhibit severe early onset hypertension correlated with impaired kidney function. However, the extra-renal mechanisms remain uninvestigated. We hypothesized that expression of CUL3Δ9 protein in smooth muscle in mice impairs endogenous wildtype CUL3 (CUL3-WT) function and causes vascular dysfunction and hypertension. We generated transgenic mice inducibly expressing CUL3Δ9 protein in smooth muscle (S-CUL3Δ9) and measured blood pressure (BP) by radiotelemetry. We assessed vascular responses in the cerebral basilar artery and aorta using a pressurized and a wire myograph, respectively. S-CUL3Δ9 mice exhibited reduced expression of endogenous CUL3WT protein compared to non-transgenic (NT) in aorta. Systolic BP was significantly increased in S-CUL3Δ9 mice (127±2 S-CUL3Δ9 vs 117±1 NT, p=0.02). Basilar artery from S-CUL3Δ9 mice exhibited significantly impaired vasorelaxation to acetylcholine (ACh) (at 100 μM: 15±4% S-CUL3Δ9 vs 65±5% NT, p<0.0001), and to the nitric oxide donor sodium nitroprusside (SNP) (at 100 μM: 59±2% S-
CUL3Δ9 vs 90±5% NT, p<0.05). Vasoconstriction to angiotensin II (Ang II), phenylephrine (PE) and to endothelin 1 (ET-1) were significantly elevated in S-CUL3Δ9 transgenic mice. Consistent with data from basilar artery, aorta from S-CUL3Δ9 transgenic mice exhibited impaired ACh-mediated relaxation (at 100 μM: 55±2% S-CUL3Δ9 vs 71±7% NT, p<0.0001). Total RhoA protein was significantly elevated in aorta of S-CUL3Δ9 transgenic mice (1.6±0.2 S-CUL3Δ9 vs 1.0±0.1 NT, P<0.05). Serotonin stimulation caused a significant increase in active RhoA in S-CUL3Δ9 aorta (1.83±0.04 S-CUL3Δ9 versus 1.52±0.06 NT, p=0.005). Preincubation with the Rho-kinase inhibitor (Y27632) restored endothelium-dependent relaxation in basilar artery and aorta of S-CUL3Δ9 mice. Ang II infusion via osmotic minipump (200 ng/kg/min) resulted in elevated BP response (Systolic BP: 147 ± 2 S-CUL3Δ9 versus 130 ± 5 NT, p=0.04) and increased aortic stiffening in S-CUL3Δ9 mice. We conclude that CUL3Δ9 acts in a dominant negative manner by interfering with CUL3-WT and contributes at least in part to hypertension via its effects on the vasculature.

L.N. Agbor: None. J. Wu: None. S.C. Ibeawuchi: None. C. Hu: None. D.R. Davis: None. H.L. Keen: None. F.W. Quelle: A. Employment; Significant; University of Iowa. C.D. Sigmund: A. Employment; Significant; University of Iowa. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA SFRN. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Significant; Carver Trust.

Funding: No

Funding Component: 049

Phosphodiesterase-3a Catalytic-domain Mutation and Hypertension

Sylvia Bähring, Medical Faculty of the CharitÃ©, Berlin, Germany; Carolin Schächterle, Max-Delbrück Ctr, Berlin, Germany; Atakan Aydin, Medical Faculty of the CharitÃ©, Berlin, Germany; Enno Klussmann, Max-Delbrück Ctr, Berlin, Germany; Friedrich C Luft Esq., Medical Faculty of the CharitÃ©, Berlin, Germany

We recently discovered phosphodiesterase-3A (PDE3A) mutations causing a 50 mm Hg increase in blood pressure and stroke >50 years, as the first non-salt form of Mendelian genetic hypertension, autosomal-dominant hypertension with brachydactyly (HTNB). The mutations cause increased PDE3A phosphorylation and higher cAMP affinity. We now have found a completely different PDE3A mutation causing a similar syndrome in a New Zealand pedigree. The mutation resides in the enzyme’s catalytic domain, results in an arginine-to-cysteine substitution, and represents a more direct mechanism of PDE3A activation. For Michaelis-Menten kinetics of cAMP hydrolysis, we transfected HEK293 cells transiently expressing Flag-tagged versions of PDE3A1, PDE3A2, or PDE3A3 mutant vs. wildtype and stimulated with forskolin and phorbol-12-myristate-13-acetate (PMA) to enhance intrinsic phosphorylation. Vmax and Km (Michaelis constant) were calculated using GraphPad Prism software to reveal the maximum cAMP turnover rate at saturated substrate concentration and the affinity of cAMP to wildtype and mutated PDE3A1, PDE3A2 and PDE3A3. For PDE3A1 hydrolytic activity (triplicate), we observed: Vmax Km Wildtype 7.5 340 Wildtype+forskolin/PMA 7.2 203 Mutant 6.6 116 Mutant+forskolin/PMA 6.3 81 The dramatically lower Km of mutant PDE3A indicates a substantially greater affinity for cAMP consistent with gain-of-function. These
data underscore the importance of PDE3A to high blood pressure by means of a different, novel genetic mechanism directly implicating the catalytic domain.

S. Bähring: None. C. Schächterle: None. A. Aydin: None. E. Klussmann: None. F.C. Luft: None.

Funding: No
Funding Component:

050

mir-192-5p in the Kidney is Protective Against the Development of Hypertension

Maria Angeles Baker, Pengyuan Liu, Yong Liu, Alison Kriegel, Kevin Regner, Mingyu Liang, Medical Coll of Wisconsin, Wauwatosa, WI

MicroRNAs (miRs) are short RNAs that primarily reduce protein abundance by base-pairing with their target mRNA. The role of most miRs in the development of hypertension remains unknown. We performed a deep sequencing analysis of miR expression in human kidney biopsies with hypertensive nephrosclerosis or without any significant injury. miR-192-5p was one of the most abundant miRs detected and was down-regulated in hypertensive nephrosclerosis. Previous studies have shown that miR-192-5p targets the beta 1 subunit of Na/K-ATPase which drives renal tubular reabsorption. We hypothesized that miR-192-5p in the kidney protects against hypertension. We used the Dahl salt sensitive (SS) rat and a congenic rat SS.13BN26 (L26) with reduced salt sensitivity as well as Mir192 knockout mice (KO) to test this hypothesis. SS rats had a decreased level of abundance of miR-192-5p in the renal cortex compared to the L26 rats (n=9, p<0.05). The protein abundance of the beta 1 subunit of Na/K-ATPase was higher in the SS rat compared to the L26 rat (n=3, p<0.05). Treatment with anti-miR-192-5p, delivered directly into the kidney through renal artery injection, in uninephrectomized L26 rats significantly exacerbated hypertension. Mean arterial blood pressure (MAP) of L26 rats treated with anti-miR-192-5p reached 151+/-5 mmHg at day 14 post anti-miR treatment and 4% NaCl (HS) diet, which was significantly higher than L26 rats treated with a control anti-miR and HS (135+/-5 mmHg, n=6 and 8, p<0.05). Tissues were collected in additional groups of rats at 9 days after anti-miR injection, which was just before MAP became significantly different between the two groups, for analysis of Na/K-ATPase activity. Na/K-ATPase activity was increased in the renal cortex of rats treated with anti-miR-192-5p compared to control anti-miR (9.8 +/-1.8µmole/min/µg vs 7.2 +/-1.3µmole/min/µg, n=5 and 6, p<0.05). Furthermore, Mir192 KO mice treated with 1µg/Kg/min of Angiotensin II and HS for 14 days exhibited an increased MAP compared to wild-type (WT) mice (190 +/-4 mmHg vs 167 +/-12 mmHg; n= 3 WT and 5 KO, p<0.05). In conclusion, miR-192, particularly miR-192-5p in the kidney, confers significant protection against the development of hypertension.


Funding: Yes
Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

051

A Panel of CRISPR/Cas9 Genome Edited Rat Models Define Quantitative Trait Nucleotides within a Novel Rat Long Non-coding RNA Implicated in Cardiovascular Disease

Xi Cheng, Harshal Waghulde, Blair Mell, Univ of Toledo Coll of Med and Life Sciences, Toledo,
This study is focused on a GWAS locus for cardiovascular disease (QT-interval) on human chromosome 17. The homologous genomic segment of this human locus was previously mapped with high resolution to <42.5 kb on rat chromosome 10. The locus in rats regulates both QT-interval and blood pressure and contains a novel long non-coding RNA (lncRNA), with a large 19bp deletion/insertion polymorphism observed between the strains used to map the locus. Characterization of this novel lncRNA using rapid amplification of cDNA ends (RACE) provided evidence for the presence of more than a single isoform of the lncRNA. To further assess the role of this locus, a panel of CRISPR/Cas9 based gene-edited ‘knockout’ models of the lncRNA was developed. The lncRNA targeted rats were developed on the genomic background of the hypertensive Dahl salt-sensitive rats and harbored varied disruptions around the critical 19bp region. The rat strains with the disrupted lncRNA sequences had a significantly elevated blood pressure compared with the controls. QT-interval is currently being examined. Overall, this is the first demonstration of a CRISPR/Cas9 based targeted gene-editing approach applied to identify a novel lncRNA as a Blood Pressure Quantitative Trait Locus.


Funding: No

Funding Component:

052

Direct Correlation Between Blood Pressure and Nephron Endowment in a Genetic Mouse Model of Hypertension

Sean P. Didion, Univ Mississippi Med Ctr, Jackson, MS; Xuexiang Wang, Rush Univ Medical Ctr, Chicago, IL; Michael R. Garrett, Univ Mississippi Med Ctr, Jackson, MS

Blood pressure high (BPH2) mice represent a novel genetic mouse model of hypertension that was derived from an 8-way cross of common inbred strains of mice. Mice derived from the original cross were segregated by blood pressure and then inbred to establish distinct mouse lines including the BPH2 line, a normotensive genetic control (blood pressure normal; BPN3) as well as a blood pressure low (BPL1) line. The goal of the present study was to test the hypothesis that the level of blood pressure in BPH2, BPN3, BPL1, and C57BL/6 (normotensive inbred control) mice correlates with nephron endowment. Systolic blood pressure (SBP) was measured in male BPH2, BPN3, BPL1, and C57BL/6 mice (n=6/group) using tail-cuff plethysmography. SBP in BPH2, BPN3, BPL1, and C57BL/6 mice averaged 152±2, 117±1; 105±4; and 107±3 mm Hg, respectively. SBP was significantly greater (P<0.05) in BPH2 mice compared to the other three groups of mice. In contrast, SBP was found to be similar (P>0.05) between BPL1 and C57BL/6 mice and significantly (P<0.05) lower than that in either BPH2 or BPN3 mice. Total kidney weight/body weight ratios were found to be similar (P>0.05) in BPH2 and BPN3 mice and significantly (P<0.05) higher than that in either BPH2 or C57BL/6 mice. Nephron number as assessed by the acid maceration technique revealed that total nephron number in BPH2, BPN3, BPL1, and C57BL/6 averaged 10,409±285, 25,333±478, 27,300±445 and 22,533±668 nephrons per kidney, respectively. There was an extremely significant correlation (R²=0.779; P<0.0001)
between nephron number and systolic blood pressure. These data provide evidence that blood pressure in a genetic mouse model of hypertension correlates with total nephron number. These findings are consistent with the emerging concept that nephron endowment at birth or progressive loss of nephrons with age or disease directly impacts blood pressure. Thus, we suggest that the BPH2 mouse can serve as an important experimental model to investigate the intrinsic relationship between nephron endowment and blood pressure.

S.P. Didion: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH R01HL107632. X. Wang: None. M.R. Garrett: None.

Funding: No

Funding Component:

053

RhoBTB1 is a Novel Gene Protecting Against Hypertension

Masashi Mukohda, Stella-Rita C. Ibeawuchi, Chunyan Hu, Ko-Ting Lu, Debbie R Davis, Deng Fu Guo, Anand R. Nair, Larry N. Agbor, Jing Wu, Kamal Rahmouni, Frederick W. Quelle, Curt D. Sigmund, Univ of Iowa Carver Coll of Med, Iowa City, IA

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand activated transcription factor regulating metabolic and vascular function. We previously reported that mice (S-DN) expressing dominant-negative PPARγ in smooth muscle cells (SMC) are hypertensive, exhibit impaired vascular relaxation and enhanced contraction, and display reduced expression of a novel PPARγ target gene, RhoBTB1. We hypothesized that RhoBTB1 may play a protective role in vascular function that is disrupted in S-DN mice and in other models of hypertension. We generated double transgenic mice (termed R+) with tamoxifen-inducible, Cre-dependent expression of RhoBTB1 in SMC. R+ mice were crossed with S-DN to produce mice (S-DN/R+) in which tamoxifen-treatment (75 mg/kg, ip, 5 days) restored RhoBTB1 expression in aorta to normal. Thoracic aorta and basilar artery from S-DN showed impaired acetylcholine (ACh)-induced endothelial-dependent relaxation, which was reversed by replacement of RhoBTB1 in SMC (thoracic aorta, 43.3±4.4 vs 74.2±1.1%, p<0.01, basilar artery, 19.9±6.7 vs 48.1±12.3%, p<0.05, n=6). Aorta from S-DN mice also displayed severely decreased sodium nitroprusside (SNP)-induced endothelial-independent relaxation with a right-shifted dose-response, which was also reversed in tamoxifen-treated S-DN/R+ mice (p<0.01, n=6). Importantly, replacement of RhoBTB1 also reversed the hypertensive phenotype observed in S-DN mice (Radiotelemetry SBP, 135.9±3.9 vs 123.7±3.0 mmHg, p<0.05, n=4). To examine if overexpression of RhoBTB1 in SMC has a protective effect on other hypertensive models, Ang-II (490 ng/min/kg) was infused in tamoxifen treated R+ mice for 2 wks. RhoBTB1 expression prevented Ang-II-induced impairment of ACh relaxation in basilar artery (17.0±8.6 in control mice vs 40.7±5.3 % in R+ mice, p<0.05, n=4) and decreased SBP (166.0±7.2 in control mice vs 133.3±5.1 mmHg in R+ mice, p<0.05, n=4). We conclude that a) loss of RhoBTB1 function explains the vascular dysfunction and hypertension observed in response to interference with PPARγ in smooth muscle, and b) RhoBTB1 in SMC has an anti-hypertensive effect and facilitates vasodilatation.

Mechanical Stretch on Endothelial Cells Promotes Monocyte Differentiation Into Immunogenic Dendritic Cells

Roxana Loperena, Wei Chen, Annet Kirabo, David G Harrison, Jose A Gomez, Vanderbilt Univ, Nashville, TN

We have shown that dendritic cells (DCs) from hypertensive mice and monocytes in humans accumulate highly reactive isolevuloglandins (isoLGs) or isoketals that adduct to protein lysines and promote T cell activation, specifically. Monocytes that traverse the endothelium have three different fates: reemerge into circulation as an activated monocyte; differentiate into macrophages or into monocyte-derived DCs. We hypothesized that human endothelial cells exposed to hypertensive mechanical stretch will promote conversion of human monocytes into immunogenic DCs. We co-cultured human aortic endothelial cells (HAECs) with monocytes from normotensive human donors and exposed the HAEC monolayer to either normal cyclical stretch (5%) or hypertensive uniaxial cyclical stretch (10%) for 48 hours. We found that co-culture of monocytes with HAECs exposed to 10% mechanical stretch markedly increased monocyte mRNA expression of the Th17 polarizing cytokines IL-6, IL-23A and IL-1β and the p22phox subunit of the NADPH oxidase compared to 5% stretch. HAECs exposed to 10% stretch promoted monocytes in culture to differentiate into DCs, as evidenced by the surface expression of DC-SIGN, CD83 and the co-stimulatory marker CD86. These monocytes also accumulated isoLG-modified proteins compared to controls (69.73 ± 5.802 vs 10.79 ± 1.854 respectively, p = 0.0001) as evidenced by intracellular staining and flow cytometry. We also co-cultured these monocytes with T cells from the same patient and examined proliferation of the latter using CFSE. Monocytes co-cultured with 10% stretched HAECs induced a 1,500-fold increase in CD4+ T cell proliferation and a 1,300-fold increase in CD8+ T cells proliferation. In addition, monocytes co-cultured with 10% stretched HAECs had a 2-fold increase in phosphorylated STAT3 expression compared to 5% stretch cultures. Conversely, monocytes-HAEC cultures exposed to 10% stretch and treated with STAT3 inhibitor, stattic, reduced their differentiation into DCs and prevented both CD4+ and CD8+ T cell proliferation. These data show that endothelial cells exposed to mechanical stretch cross-talk with monocytes to promote differentiation into activated DCs potentially via the STAT3 pathway.


Funding: No

Funding Component:
Benefit of Mineralocorticoid Receptor Antagonism in Acute Kidney Injury: Role of Smooth Muscle Rac1

Jonatan Barrera-Chimal, Gwenan Andrée-Grégoire, Sonia Prince, INSERM U1138, Paris, France; Peter Kolkhof, BAYER Pharma AG, Cardiology Res, Wuppertal, Germany; Vincent Sauzeau, INSERM U1087, Nantes, France; Frederic Jaisser, INSERM U1138, Paris, France

Introduction: Renal ischemia/reperfusion (IR) is a major cause of acute kidney injury (AKI). The benefit of novel non-steroidal MR antagonists such as finerenone in the IR context has not been evaluated and the mechanisms underlying the benefit of MR antagonism remain unclear. Objectives: To test the efficacy of finerenone in ischemic AKI and to evaluate the specific contribution of the MR expressed in endothelial or smooth muscle cell (SMC) in renal IR injury. Methods: We included 18 male C57/B6 mice that were divided in: sham, renal ischemia for 20 min and IR plus treatment with finerenone (10 mg/kg) by gavage once a day at -48, -24 and -1 h before IR. Alternatively, MR inactivation in endothelial cells (MRendoKO mice /Vecadh-cre) or in smooth muscle cells (MRSMCKO mice/SMA-cre) was induced in 3-month-old mice. Sham surgery or bilateral renal IR for 20 min was performed and mice were studied 24 h after reperfusion. Primary rat SMC cultures were used to assess the signaling pathways modulated by MR. Results: In C57/B6 WT, MRfl/fl and MRendoKO mice, IR induced kidney dysfunction and tubular injury. After IR, Finerenone-treated mice and the MRSMCKO mice presented normal renal function and a significant reduction of histological alterations, while MRendoKO mice were not protected. The benefit of finerenone and MR KO in SMC was associated with reduced oxidative stress-mediated lipid peroxidation as compared to MRfl/fl or WT mice. In aldosterone-stimulated rat SMC, we observed a 100% increase in hydrogen peroxide production and a 2-fold increase in Rac1 activity; MR and Rac1 antagonism blunted these effects. Moreover, mice deficient of Rac1 in SMC were also protected against ischemic AKI. Conclusion: Finerenone limits renal injury induced by IR. Moreover, genetic deletion of MR in SMC only has similar effects. This benefit was associated with reduced oxidative stress, by affecting oxidative stress production via SMC Rac1.

J. Barrera-Chimal: None. G. André-Grégoire: None. S. Prince: None. P. Kolkhof: A. Employment; Modest; Employee at Bayer Pharma AG. V. Sauzeau: None. F. Jaisser: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Part of the work was supported by a grant from Bayer.

Funding: No

Funding Component:

Isolevuglandin-modified Phosphatidylethanolamines Generate Hypertension and Vascular Dysfunction in Mice

Arvind K Pandey, Hana A Itani, Liang Xiao, Annet Kirabo, Sergey Dikalov, Sean Davies, David G Harrison, Vanderbilt Univ, Nashville, TN

Oxidative stress is an important contributor to hypertension. Our group has shown that oxidative stress generates isolevuglandins (isoLG) that modify proteins and that these isoLG-modified proteins seem to act as neoantigens to promote hypertension. In addition to proteins, isoLG can also modify phosphatidylethanolamines (PEs). IsoLG-
modified PE (IsoLG-PE) can in turn activate immune cells via reactions with toll-like receptors and downstream pathways including NFκB. The role of isoLG-PE in hypertension has not yet been defined. We have previously shown that the enzyme N-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD) cleaves isoLG-PE, and mice lacking this enzyme have elevated cellular levels of isoLG-PE. To assess whether loss of NAPE-PLD and resulting increase in isoLG-PE contributes to hypertension, we infused wild type (WT) and NAPE-PLD−/− mice with low dose Ang II. We found baseline blood pressure was elevated in NAPE-PLD−/− mice compared to WT mice (128 ± 3 vs 111 ± 2 mmHg, p = 0.005) and that the increase in BP to low dose Ang II infusion (140 ng/kg/min) was augmented in NAPE-PLD−/− compared to WT mice (146 ± 9 vs 127 ± 4 mmHg, p=0.045). Endothelium-dependent vascular relaxation of the mesenteric arterioles to acetylcholine was impaired in NAPE-PLD−/− compared to WT mice (maximum relaxation of 43% ± 6% vs 53% ± 5%, p= 0.004), but endothelium-independent responses to sodium nitroprusside were similar in both groups (82% ± 2% in NAPE-PLD−/− mice vs 81% ± 4% in WT mice). Aortic adventitial collagen content by planimetry was likewise increased in NAPE-PLD−/− versus WT mice following low-dose Ang II. These studies in mice lacking NAPE-PLD suggest that in addition to exerting its effects via protein modification to form neo-antigens, the reaction of isoLG with PE and related phospholipids can augment hypertension, alter endothelium-dependent vasodilatation and promote vascular fibrosis.


Funding: No

Funding Component:

Nox5 Induces Vascular Dysfunction and Arterial Remodelling Independently of Blood Pressure Elevation in Ang II-infused Nox5-expressing Mice

Augusto C Montezano, Adam P Harvey, Francisco J Rios, Maria Dulak-Lis, Wendy Beatie, Laura McPherson, Inst of Cardiovascular and Medical Sciences - Univ of Glasgow, Glasgow, United Kingdom; Chet E Holterman, Christopher R Kennedy, Kidney Res Ctr - Ottawa Hosp Res Inst, Ottawa, ON, Canada; Rhian M Touyz, Inst of Cardiovascular and Medical Sciences - Univ of Glasgow, Glasgow, United Kingdom

Nox5 is a unique Ca2+-sensitive Nox isoform that is expressed in human vascular smooth muscle cells (VSMC). Although Nox5 has been implicated in diabetic nephropathy, its role in vascular function and development of hypertension remain unclear. Nox5 is not expressed in rodents, and accordingly we generated humanised Nox5 mice with Nox5 expressed in a VSMC-specific manner (Nox5SM22). Control (wild-type) and Nox5SM22 mice were infused with Ang II (600 ng/Kg/day). Blood pressure (BP) was assessed by tail-cuff. Vascular function and structure of resistance arteries were measured by myography. Ang II increased BP in WT (182.5±10 mmHg) and Nox5SM22 (173.1±5 mmHg) with no significant differences. Ang II increased the maximal contraction to U46619 (thromboxane A2 mimetic) in WT (115.8±2 vs untreated: 101.4±2%) and Nox5SM22 (121.3±3 vs untreated: 99.1±2%) (p<0.05) and induced endothelial dysfunction in all groups. Fasudil-

057
induced relaxation was impaired by Ang II in WT (102.7±6 vs untreated: 166.6±8%, p<0.05) but not further impaired in Nox5SM22 mice (114.9±6 vs untreated: 111.3±11%). Ang II increased cross-sectional area (CSA) and lumen diameter; while in Nox5SM22 mice, Ang II increased wall thickness, wall-to-lumen ratio, CSA and decreased lumen diameter, with associated increased vascular stiffness. Our findings indicate that in mice expressing human Nox5 in VSMCs, endothelium-dependent relaxation is impaired, fasudil-mediated vasodilation is attenuated and vessels undergo exaggerated hypertrophic inward remodelling with increased stiffness; processes that occur independently of BP elevation. These data suggest an important role for Nox5 in Ang II-induced vascular dysfunction and remodeling, but not in the development of hypertension. Moreover, we identify Rho kinase as a putative target for Nox5-induced vascular injury. We provide novel insights into Nox5 vascular biology and demonstrate that vascular Nox5 actions are dissociated from BP effects.


Funding: No

Funding Component:

058

Transforming Growth Factor β1 Antagonizes Npr1 Expression and Vascular Signaling: Role of Transcription Factor δEF1

Transforming Growth Factor β1 Antagonizes Npr1 Expression and Vascular Signaling: Role of Transcription Factor δEF1

Anagha Sen, Prerna Kumar, Sarah H. Lindsey, Prasad V. Katakam, Meaghan Bloodworth, Kailash N. Pandey, Tulane Univ, New Orleans, LA

The objective of the present study was to examine the repressive effect of transforming growth factor beta 1 (TGF-β1) in the regulation of Npr1 (coding for guanylyl cyclase/natriuretic peptide receptor-A; GC-A/NPRA) gene expression and vascular signaling. The rat thoracic aortic vascular smooth muscle cells (RTASMC) and denuded aortic rings were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and treated with TGF-β1 in a time-and dose-dependent manner. Treatment with TGF-β1 decreased Npra mRNA and protein levels by 62% (0.42 ± 0.05 vs. control, 0.9 ± 0.02, p < 0.01) and 55% (9603 ± 860 vs. control, 22211 ± 1449, p < 0.01), respectively. TGF-β1 treatment significantly increased delta EF1 (δEF1) protein expression by 2.4-fold (907.9 ± 36.5 vs. control, 378.5 ± 10.3; p < 0.001) and enhanced its recruitment to Npr1 promoter. TGF-β1-treated RTASMCs and denuded aortic rings showed significant increases in α-smooth muscle actin (α-SMA) and collagen type 1 alpha 2 (COL1A2) protein expression, which were markedly attenuated by ANP treatments. The TGF-β1-pretreated cells showed 2.6-fold increase in α-SMA (control, 1523 ± 143, TGF-β1, 3997 ± 182 and TGF-β1 + ANP, 2172 ± 135) and 3.4-fold increase in COL1A2 (control, 1250 ± 77, TGF-β1, 4234 ± 110 and TGF-β1 + ANP, 1546 ± 57), respectively. In ex vivo experiments of denuded-aortic rings, TGF-β1 decreased Npr1 mRNA and protein levels by 62% (0.39 ± 0.06 vs. control 1.10 ± 0.01) and 70% (2609 ± 69 vs. control 5775 ± 123), respectively, and significantly (p < 0.0) increased the expression of TGF-β1-responsive proteins, namely α-SMA (2.6-fold) and COL1A2 (3.1-fold). Treatment with increasing concentrations of ANP (IC50=6x10-9M), relaxed denuded aortic rings contracted with prostaglandin F2α (PGF2α); however, pretreatment with TGF-β1 significantly attenuated ANP-mediated vascular relaxation after PFG2α contraction (ANP-treated, 68.68 ±
9.4 vs. TGF-β1 + ANP-treated 88.85 ± 4.7). The endothelium-intact vessels were not affected by TGF-β1 incubation. Together, the present results suggest that an antagonistic cascade exists between TGF-β1 pathways and ANP/NPRA signaling, which might be critical in the vascular remodeling and regulation of hypertension and cardiovascular homeostasis.


Funding: No

Funding Component:

059

**Kidney Specific DPP4 Knockdown Prevents Ang II/DOCA Salt Injury and Proteinuria**

Jianzhong An, Qiuer Liu, Cassandra Smith, Ravi Nistala, Univ of Missouri-Columbia, Columbia, MO

Dipeptidyl peptidase 4 (DPP4) inhibition is widely used for glycemic control in type 2 diabetes mellitus (T2DM) subjects. The Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus - Thrombolysis in Myocardial Infarction 53 (SAVOR-TIMI 53) trial showed that DPP4 inhibition improves proteinuria in type 2 DM (T2DM) subjects with chronic kidney disease (CKD). Our lab and others have observed that kidney DPP4 activation is associated with proteinuria in conditions of RAAS activation including obesity. However, it is not clear if kidney specific DPP4 directly plays a role in kidney injury. To test our hypothesis, right nephrectomy (UNx) mice were subjected to 1) sham surgery 2) left renal vein injection with lentivirus carrying DPP4 shRNA (UNx+DPP4) 3) scramble shRNA (UNx+SCRM) and the animals were allowed to recover for 2-3 wks. A 4th group of non-UNx mice were added as additional controls. Next, angiotensin II (Ang II, 1000ng/kg/min) plus deoxycorticosterone (DOCA, 50mg) plus 0.9% NaCl (salt) or saline only was given for 2 wks. Ang II/DOCA salt caused heart hypertrophy in all groups when compared to saline infusion (p<0.05). DPP4 knockdown reduced albuminuria (UNx+Ang II/DOCA salt, 80.18±15.3 ug/mg Cre; UNx+DPP4+Ang II/DOCA salt, 25.76±19.3 ug/mg Cre; UNx+SCRM+Ang II/DOCA salt 100.1±17.6 ug/mg Cre, p<0.05). Flow cytometry analysis showed that DPP4 knockdown mitigated activation of CD4+ T cells by Ang II/DOCA salt as indicated by reduced surface expression of DPP4 on CD4+, CD44+CD127+ central memory and CD44+CD127- effector cells. In addition, DPP4 knockdown prevented Ang II/DOCA salt-mediated infiltration of Ly6ChiCD11bhiF4/80lo macrophages and decreased the Ly6C-CD11bloF4/80hi resident dendritic cells. DPP4 knockdown suppressed Ang II/DOCA salt mediated activation of pro-fibrotic markers, Col1A and CTGF. Lastly, DPP4 knockdown suppressed glomerulomegaly, mesangial expansion and interstitial inflammation on trichrome stain. In summary, RAAS activation is a prominent mechanism for kidney injury in T2DM and obese subjects. Kidney DPP4 is an important effector of RAAS mediated kidney inflammation, activation of pro-fibrotic markers and proteinuria, thereby implicating DPP4 in CKD initiation and progression.

J. An: None. Q. Liu: None. C. Smith: None. R. Nistala: A. Employment; Significant; Dialysis Clinics Inc.. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Dialysis Clinics Inc..

Funding: No

Funding Component:

060
Tauroursodeoxycholic Acid (TUDCA) Prevents High Salt-Induced Renal Damage in ETβ Deficient Rats in a Blood Pressure-Independent Manner

Carmen De Miguel, Randee Sedaka, Andrew Abad, Janet L. Hobbs, David M. Pollock, Jennifer S. Pollock, Univ of Alabama at Birmingham, Birmingham, AL

The molecular mechanisms by which the vasoactive peptide endothelin-1 (ET-1) leads to high salt-induced renal damage remain uncertain. These studies were designed to determine if the ET system induces renal damage by increasing renal cellular stress. ETβ deficient (ETβ def) and transgenic (TG) control rats were placed on normal (NSD, 1% NaCl) or high salt (HSD, 8% NaCl) diet for 3 weeks, and received daily i.p. injections of the cellular stress reliever tauroursodeoxycholic acid (TUDCA, 400 mg/kg/day) or vehicle. Blood pressure was monitored by telemetry. At the end of the study, renal inflammation, apoptosis and markers of kidney damage were assessed. In ETβ def rats, HSD significantly increased blood pressure (NSD vs. HSD: 131.2±1.1 vs. 153.9±5.9 mmHg, p<0.05), albuminuria, excretion of cortical tubular injury markers (NSD vs. HSD: KIM-1: 14.2±3.4 vs. 104.1±20.6 pg/day; NGAL: 43.6±17.6 vs. 215.6±27.7 pg/day; n=4-7/group; p<0.05), cortical T cell infiltration (5.7±0.3 vs. 17.4±4.5 CD3+ cells/field; p<0.05) and cortical apoptosis (4.0±1.0 vs. 18.0±4.2 TUNEL+ cells/field; n=3/group; p<0.05). No changes were seen in TG controls on HSD. TUDCA treatment prevented HSD-induced cortical damage in ETβ def rats, as indicated by reduced excretion of kidney injury markers (albumin: 2.3±1.5 ng/day; KIM-1: 55.7±13.8 pg/day; NGAL: 114.2±18 pg/day; p<0.05), cortical T cell infiltration (10.8±0.5 CD3+cells/field; p<0.05) and cortical tubular apoptosis (1.9±0.3 TUNEL+ cells/field; p<0.05). TUDCA had no effect on renal medullary apoptosis or blood pressure. Nephrin excretion remained elevated in both genotypes despite TUDCA treatment. In conclusion, loss of ETβ receptor leads to exaggerated salt-mediated renal cortical damage that is attenuated by treatment with TUDCA. These results highlight the protective role of ETβ receptors against the development of renal damage, especially in renal cortex. Funded by NIH T32 DK007545 to CDM and P01 HL95499 and P01 HL69999 to DMP and JSP.

C. De Miguel: None. R. Sedaka: None. A. Abad: None. J.L. Hobbs: None. D.M. Pollock: None. J.S. Pollock: None.

Funding: No

Funding Component:

061

Cell Fate Changes During Tubular Damage and Regeneration in the Mouse Kidney

Vidya K Nagalakshmi, Minghong Li, R. Ariel Gomez, Maria Luisa S. Sequeira-Lopez, Univ of Virginia, Charlottesville, VA

Tubular degeneration, loss of renal tubules and interstitial fibrosis due to kidney injury lead to chronic renal disease and hypertension. Using a partial unilateral ureteral obstruction (pUUO) model in neonatal mice, we analyzed the fate cell changes that occur during obstruction and during recovery following the release of UUO. We traced the fate of cells derived from the renal stroma, cap mesenchyme and ureteric bud epithelium using Foxd1-Cre, Six2-Cre and HoxB7-Cre mice respectively, crossed with double fluorescent reporter (mT/mG) mice. pUUO was performed 24-36h after birth (n=84). In a group of pups (n=37), the obstruction was released after seven days. Sham operated animals (n=35) were used as controls. Lineage tracing revealed that Foxd1-derived interstitial
pericytes acquired α-smooth muscle actin expression and underwent significant expansion due to pUUO (fibrotic area 91.06+/-6.77 %). Release of obstruction resulted in complete resolution of fibrotic areas (0.00%; p<0.005). Further, loss of Six2-derived cells at the glomerular-tubular junction in pUUO kidneys resulted in the formation of atubular glomeruli (39%). Atubular glomeruli were not observed after release. In addition, a significant loss of HoxB7-derived collecting duct tubules was observed during pUUO. Most collecting ducts recovered following release. Our study indicates that obstruction leads to significant tubular damage, expansion of interstitial pericytes, fibrosis, tubular loss and formation of atubular glomeruli. The striking recovery observed after release of ureteral obstruction suggests a reversal of cell fate changes and tubular regeneration. Elucidation of the cellular and molecular mechanisms mediating these events may be of use in the design of strategies for the prevention and/or treatment of kidney diseases and secondary hypertension.

V.K. Nagalakshmi: None. M. Li: None. R.A. Gomez: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, NIDDK, NHLBI. M.S. Sequeira-Lopez: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, NIDDK.

Funding: No

Funding Component:

062

**Regulation of Nephron Afferent Arteriole Resistance in Obesity: Role of Connecting Tubule Glomerular Feedback**

**Sumit R Monu, Mani Maheshwari, Hong Wang, Ed Peterson, Oscar Carretero, Henry Ford Hosp, Detroit, MI**

In obesity, renal damage is caused by increase in renal blood flow (RBF), glomerular capillary pressure (Pgc), and single nephron glomerular filtration rate but the mechanism behind this alteration in renal hemodynamics is unclear. Pgc is controlled mainly by the afferent arteriole (Af-Art) resistance. Af-Art resistance is regulated by mechanism similar to that in other arterioles and in addition, it is regulated by two intrinsic feedback mechanisms: 1) tubuloglomerular feedback (TGF) that causes Af-Art constriction in response to an increase in sodium chloride (NaCl) in the macula densa, via sodium–potassium-2-chloride cotransporter-2 (NKCC2) and 2) connecting tubule glomerular feedback (CTGF) that causes Af-Art dilatation and is mediated by connecting tubule via epithelial sodium channel (ENaC). CTGF is blocked by the ENaC inhibitor benzamil. Attenuation of TGF reduces Af-Art resistance and allows systemic pressure to get transmitted to the glomerulus that causes glomerular barotrauma/damage. In the current study, we tested the hypothesis that TGF is attenuated in obesity and that CTGF contributes to this effect. We used Zucker obese rats (ZOR) while Zucker lean rats (ZLR) served as controls. We performed *in-vivo* renal micropuncture of individual rat nephrons while measuring stop-flow pressure (PsF), an index of Pgc. TGF response was measured as a decrease in PsF induced by changing the rate of late proximal perfusion from 0 to 40nl/min in stepwise manner. CTGF was calculated as the difference of PsF value between vehicle and benzamil treatment, at each perfusion rate. Maximal TGF response was significantly less in ZOR (6.16 ± 0.52 mmHg) when compared to the ZLR (8.35 ± 1.00mmHg), p<0.05, indicating TGF resetting in the ZOR. CTGF was significantly
higher in ZOR (6.33±1.95 mmHg) when compared to ZLR (1.38±0.89 mmHg), p<0.05. When CTGF was inhibited with the ENaC blocker Benzamil (1μM), maximum Psf decrease was 12.30±1.72 mmHg in ZOR and 10.60 ± 1.73 mmHg in ZLR, indicating that blockade of CTGF restored TGF response in ZOR. These observations led us to conclude that TGF is reset in ZOR and that enhanced CTGF contributes to this effect. Increase in CTGF may explain higher renal blood flow, increased Pgc and higher glomerular damage in obesity.

Funding: No
Funding Component:

063

Role of Ribosomal S6 Kinase 2 in Vascular Myogenic Tone and Blood Pressure Regulation

Mykhaylo Artamonov, Miranda Good, Ko Momotani, Brant Isakson, Robert Carey, Thu H. Le, Avril V. Somlyo, Univ of Virginia, Charlottesville, VA

Smooth muscle (SM) contractile force is generated through phosphorylation of the myosin regulatory light chain (RLC20) by the myosin light chain kinase. However, emerging evidence also suggests that additional Ser/Thr kinases may contribute to the pathways that regulate SM contractility. Recently, we published that the 90 kDa ribosomal S6 kinase (RSK), extensively studied in cancers of epithelial origin, is a major new player in the regulation of SM contractility. RSKs are unusual kinases having two catalytic domains separated by a linker having multiple phosphorylation sites and are activated by a cascade of ERK phosphorylations and by PDK, which phosphorylates RSK at Ser227 resulting in transition to a fully active enzyme. The thromboxane agonist, U46619 leads to activation of ERK1/2 and PDK1 in SM. We find that 4th order mesenteric arteries are significantly more dilated at a pressure of 80 mm Hg in Rsk2−/− mice compared to wild-type (WT) (2.0±0.8 vs 12.8±4.5 %, p<0.05, respectively). The contractile response to dose-dependent phenylephrine stimulation is significantly smaller in the absence of RSK2, p<0.0001 two-way ANOVA, while EC50 for phenylephrine contractions did not differ. Using isolated arcades of mesenteric vessels, phosphorylation of myosin RLC20 and myosin phosphatase regulatory subunit and RSK Ser227 (PDK site) and Thr 577 (ERK site) increased with in 30secs following an increase in intraluminal pressure to 80 mm Hg to induce myogenic tone development. Mean baseline systolic blood pressure (SBP, mmHg) was significantly lower in Rsk2−/− animals (WT=107.4±1.6 and RSK2−/− =95.8±2.2, n=23, p< 0.0002). In view of the lower SBP in the Rsk2−/− animals, we examined the potential role for RSK signaling in the kidney, since RSK2 is known to phosphorylate NHE3. By immunohistochemistry, we show for the first time the expression of RSK2 in human kidney tubules. Lithium excretion, moles/kg/day, was increased in Rsk2−/− mice compared to WT (21.7±3.7 vs 48.6±7.9, respectively), indicative of defective renal Na+ reabsorption. Taken together, RSK2 signaling in vascular SM and the kidney is a novel mechanism for the regulation of SM contractility and of BP. RSK2 may be a potential selective target in hypertension.

Funding: No
Funding Component:
Role of Sphingosine-1-phosphate 2 Receptor in Renal Microvascular Function of Renal Ischemia-reperfusion Rats

Zhengrong Guan, Edward W Inscho, Univ of Alabama at Birmingham, Birmingham, AL

Renal ischemia-reperfusion (IR) induced acute kidney injury (IR-AKI) is accompanied by increased renal vascular resistance and a dramatic decline in glomerular filtration rate. Sphingosine-1-phosphate (S1P), a bioactive sphingolipid metabolite, plays a critical role in IR-AKI. Our recent studies established that exogenous S1P is a potent vasoconstrictor in rat afferent arterioles. We postulated that renal IR enhanced sensitivity of afferent arterioles to S1P-induced vasoconstriction via S1P2 receptor activation. The afferent arteriole response to S1P was assessed using the in vitro blood-perfused juxtamedullary nephron preparation. IR rats were induced by 60 min bilateral renal artery occlusion followed by 24 hours reperfusion. Baseline arteriolar diameter decreased significantly in IR (11.9±0.7 vs. 14.7±0.5 µm in sham, n=7, P<0.05). Exogenous S1P evoked concentration-dependent vasoconstriction in sham rats. Increasing S1P (10^{-10}-10^{-5} M) decreased diameter to 99±1, 95±2, 89±2, 80±3, 58±4, and 34±2% of baseline (n=7), respectively. Renal IR shifted the S1P vasoconstrictor profile to the left. Baseline arteriolar diameter declined to 93±1, 87±1, 76±1, 64±5, 46±5 and 35±4% of baseline (n=7, P<0.05 vs sham at 10^{-10}-10^{-7} M S1P). S1P2 receptor blockade with JTE-013 markedly increased baseline diameter by 51±13% in IR (P<0.05, vs. 11±3% in shams, n=3-4), suggesting that endogenous S1P exerts a greater influence on arteriolar tonic in IR than in sham. JTE-013 also markedly attenuated the enhanced vasoconstriction to S1P in IR rats. Arteriolar diameter averaged 112±2, 110±1, 114±3, 102±6, 81±6 and 61±10% of control in response to increasing S1P (P<0.05 vs. JTE-013 untreated-IR) similar to JTE-013 treated sham. In contrast, arteriolar responses to Ang II, endothelin-1 or the S1P precursor, sphingosine were unaltered in IR rats. S1P content increased from 1.3±0.4 to 1.9±0.4 pmol/mg protein (~45%) in the cortical tissue of IR rats and 2.2±0.4 to 3.4±0.2 pmol/mg protein (~55%) in the medullary tissue but reduced the plasma S1P concentration from 2.5±0.3 to 1.3±0.1 µM (~50%, P<0.05, n=3). These data establish that renal IR increases sphingolipid metabolites in rat kidneys and enhances renal microvascular S1P signaling, probably via S1P2 receptor activation.

Z. Guan: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, National Scientist Development Grant (10SDG3770010), AHA, Grant-in-Aid (15GRNT25240015). E.W. Inscho: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH DK044628, NIH HL074167, NIH HL098135, NIH HL095499.

Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

Overexpression of an Intracellular Angiotensin II Fusion Protein Selectively in the Mitochondria of the Proximal Tubules Elevates Blood Pressure in Mice via AT1a Receptor-mediated Mitochondrial Respiratory and Glycolysis Stress

Xiao C Li, Univ of Mississippi Medical Ctr, Jackson, MS; Manoocher Soleimani, Univ of Cincinnati, Cincinnati, OH; Hoang Nguyen, Univ of Mississippi Medical Ctr, Jackson, MS; Hong Li,
An intracrine mitochondrial renin-angiotensin system (RAS) has recently been identified in various animal and human tissues, but whether the mitochondrial RAS plays a physiological role in the regulation of blood pressure remains unknown. The present study tested whether overexpression of an intracellular angiotensin II fusion protein, ECFP/ANG II, selectively in the mitochondria of the proximal tubules alters blood pressure, and whether the effects may involve AT1a receptors and the Na+/H+ exchanger 3 (NHE3). An adenoviral vector encoding ECFP/ANG II, a mitochondria targeting sequence, and the sglt2 promoter, Ad-sglt2-mito-ECFP/ANG II, was constructed for proximal tubule- and mitochondria-specific overexpression for 2 weeks. In adult male C57BL/6J mice, overexpression of mito-ECFP/ANG II in the mitochondria of the proximal tubules increased systolic blood pressure (SBP) significantly (Control: 116 ± 3 vs. mito-ECFP/ANG II: 128 ± 3 mmHg; p<0.01, n=15). The blood pressure-increasing effect of Ad-sglt2-mito-ECFP/ANG II was blocked in proximal tubule-specific AT1a-KO mice (Control: 105 ± 2 vs. mito-ECFP/ANG II: 104 ± 4 mmHg; n.s., n=7), or in proximal tubule-specific NHE3-KO mice (Control: 108 ± 3 vs. mito-ECFP/ANG II: 107 ± 3 mmHg; n.s., n=13), respectively. In further experiments, mouse proximal tubule cells were transfected with Ad-sglt2-mito-ECFP/ANG II for 48 h and treated with the AT1 blocker losartan (10 μM) or the AT2 blocker PD123319 (10 μM) to measure mitochondrial respiratory and glycolytic function using Seahorse XF Cell Mito and XF Glycolysis Stress Tests. The mito-ECFP/ANG II expression was robust and colocalized with MitoTracker® Red FM. Overexpression of mito-ECFP/ANG II markedly increased oxygen consumption rate (OCR) (Control: 139.4 ± 9.2 vs. mito-ECFP/ANG II: 236.3 ± 12.6 pmol/min; p<0.01, n=12) and extracellular acidification rate (ECAR) (Control: 8.8 ± 0.6 vs. mito-ECFP/ANG II: 11.8 ± 1.2 mPH/min; p<0.01, n=12), respectively. Losartan blocked the effects of mito-ECFP/ANG II on OCR and ECAR, whereas PD123319 had no effect. We conclude that intracellular ANG II may activate AT1 receptors in the mitochondria of the proximal tubules to alter mitochondrial respiratory and glycolytic function and arterial blood pressure.


Funding: No
cells, we studied As4.1 cells, kidney tumor cells that express renin constitutively, and native renin cells sorted from the kidneys of Ren1cKO-YFP* mice. In these mice, the renin promoter drives YFP expression thus marking the renin cells. We used genome-wide ChIP-Seq for Med1 (subunit 1 of the Mediator complex), H3K27Ac (active enhancers) and Pol II (to visualize putative genomic areas undergoing transcription). The ROSE algorithm we used to ascertain super-enhancers. Chromatin accessibility genome-wide was assessed using ATAC-Seq. The results were compared to twenty-one other cell types that do not express renin. In As4.1 cells, we identified 14,871 enhancers based on H3K27Ac. Of those, 888 were classified as super-enhancers. The Med1 signal in As4.1 cells showed a SE localized 5kb upstream the Ren1 gene, which was ranked at position 25 among other SEs. The H3K27Ac signal showed highest occupancy in the same region. ChIP-Seq for H3K27Ac in YFP+ cells showed 211 SEs of 2,987 peaks. The SE for the renin gene possessed the highest signal and ranked number 1, indicating its importance in renin cells. One hundred and thirteen SEs were unique to renin cells, including the SE associated with the renin gene. ATAC-Seq signals overlapped with the renin SE and the classical enhancer indicating that the chromatin was accessible for transcription. In summary, renin-expressing cells possess distinct repertoires of unique enhancers and super-enhancers that acting in concert are likely to determine the renin phenotype.

M.F. Martinez: None. S. Medrano: None. M. Oka: None. E.S. Pentz: None. A.W. Dickerman: None. M. Adli: None. M.S. Sequeira-Lopez: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, NIDDK, NHLBI.

Funding: No
Funding Component:

067

Unexpected Actions of the Endothelial-EP4 Receptor for PGE2 to Promote Hypertension

Ting Yang, Marcela Herrera, Matthew A Sparks, Michael Manning, Duke Univ, Durham, NC; Beverly H Koller, Univ of North Carolina, Chapel Hill, NC; Thomas M Coffman, Duke Univ, Durham, NC

Prostaglandin E2 (PGE2) is a major prostanoid produced by the kidney with vasodilator and natriuretic actions and its actions are mediated by four distinct E-prostanoid (EP) receptor isoforms: EP1-EP4. The EP4 receptor (EP4R) has multiple actions that could impact blood pressure (BP) by triggering macula densa stimulation of renin, inducing vasodilation, and inhibiting epithelial sodium transport. Accordingly, we examined the role of EP4R on BP regulation by generating EP4R-deficient mice. Because deletion of EP4R in utero causes peri-natal mortality due to persistent patent ductus arteriosus, we carried out conditional deletion using an EP4flox/flox mouse line. We first generated mice completely lacking EP4R in all tissues (TBKO) using a tamoxifen-inducible transgene driving Cre expression in all tissues. Resting mean arterial pressure (MAP) measured by radiotelemetry tended to be elevated in TBKOs compared to controls (106±2 vs 111±2 mmHg; p=0.06). In addition TBKOs showed exaggerated salt sensitivity and enhanced hypertensive response to chronic Ang II infusion compared to controls (MAP increase: 25±3 vs. 37±2 mmHg; p<0.05). To determine whether altered BP responses in the TBKOs were due to elimination of EP4R-dependant actions in
vascular smooth muscle cells (VSMCs) or in endothelial cells (ECs), we generated mice lacking EP₄R in VSMCs (SMKO) or ECs (ECKO) using EP₄flox/flox and transgenic mice with tamoxifen-inducible expression of Cre limited to VSMCs or ECs. Resting MAP in SMKO mice was significantly reduced compared to controls (109±1 vs. 104±2 mmHg; p<0.05), but salt sensitivity and Ang II-dependent hypertension were unaffected. Although no statistically significant differences in baseline MAP or salt sensitivity were observed between ECKOs and controls, the hypertensive response to AngII infusion was significantly reduced in ECKOs (MAP increase: 31±3 vs 24±2 mmHg; p<0.05). In summary, our work suggests a complex role for PGE₂ acting via its EP₄R in BP regulation, with a major effect to promote resistance to hypertension, apparent in the TBKOs. However, we have also uncovered an unexpected and opposing effect of EP₄R in endothelium to promote hypertension.


Funding: No

Funding Component:

068

Endothelin-1 Exaggerates Type-1 Diabetes-accelerated Atherosclerosis Through NADPH Oxidases 1 and 4


Objective: NADPH oxidase (NOX) 1 but not NOX4-dependent oxidative stress plays a role in diabetic vascular disease, including atherosclerosis. Endothelin (ET)-1 has been implicated in diabetes-induced vascular complications. We showed that crossing mice overexpressing ET-1 selectively in endothelium (eET-1) with apolipoprotein E knockout (Apoe⁻/⁻) mice exaggerated high-fat diet-induced atherosclerosis in part by increasing oxidative stress. We hypothesized that ET-1 overexpression in the endothelium would exaggerate diabetes-accelerated atherosclerosis through a mechanism involving NOX1 but not NOX4.

Methods: Six-week-old male Apoe⁻/⁻ mice, eET-1/Apoe⁻/⁻ and eET-1/Apoe⁻/⁻ mice deficient in Nox1 (eET-1/Apoe⁻/⁻/Nox1⁻/⁻) or Nox4 (eET-1/Apoe⁻/⁻/Nox4⁻/⁻) were rendered diabetic with 55 mg/kg/day streptozotocin (STZ) IP injections for 5 days and studied 14 weeks later. Endothelial function and vascular remodeling were assessed in mesenteric arteries (MA) using pressurized myography. Aortic atherosclerotic lesions were quantified using Oil Red O staining. Plasma cholesterol, HDL and triglycerides were measured.

Results: Diabetic Apoe⁻/⁻ mice presented an impaired endothelium-dependent vasodilatory response to acetylcholine, which was not observed in diabetic eET-1/Apoe⁻/⁻, eET-1/Apoe⁻/⁻/Nox1⁻/⁻ or eET-1/Apoe⁻/⁻/Nox4⁻/⁻ mice (Eₘₐₓ: 20±6 vs 99±1, 98±1 and 100±0%). ET-1
Overexpression caused a 1.8-fold increase in MA media/lumen of diabetic Apoe−/− mice (5.3±0.3 vs 2.9±0.2%), which was further increased 1.2-fold by Nox4 (6.4±0.3%) but not Nox1 knockout (5.5±0.3%). ET-1 overexpression exaggerated >2-fold the atherosclerotic lesion area in the aortic sinus in diabetic Apoe−/− mice (plaque area [x10^5 µm^2]: 5.3±0.5 vs 2.9±0.6), which was reduced ~40% by Nox1 and Nox4 knockout (plaque area [x10^5 µm^2]: 3.3±0.6 and 3.6±0.6). Plasma triglycerides were unaffected by ET-1 overexpression but reduced by Nox1 (2.2±0.4 vs 3.4±0.3 mmol/L) and Nox4 knockout (1.8±0.4 mmol/L). Plasma HDL and cholesterol were similar between groups.

**Conclusions:** Increased levels of ET-1 exaggerate diabetes-accelerated atherosclerosis through NOX1 and NOX4, despite paradoxically improving endothelium-dependent relaxation in small arteries.


**Funding:** No

**Funding Component:**

069

**β-Catenin Signaling Contributes to Angiotensin II-Induced Hypertension via Activation of Sodium Transporters in the Distal Nephron**

Xiaohan Lu, Kexin Peng, Fei Wang, Inst of Hypertension, Guangzhou, China; Tianxin Yang, Univ of Utah, Salt Lake City, UT

β-Catenin signaling plays an important role in regulation of development as well as tissue homeostasis. Recently, we have shown that this pathway is involved in regulation of collecting duct (CD) function. Here, we examined the role of β-catenin signaling in AngII-induced hypertension. C57/BL6 mice were administered for 7 d with vehicle, AngII alone (300 ng/kg/min) or in combination with an inhibitor of β-catenin pathway, ICG-001 (50 mg/kg/d) both via an osmotic mini-pump. Radio telemetry demonstrated that ICG-001 effectively attenuated AngII-induced hypertension (MAP on day 7: 112.5 ± 2.02 in Control group vs. 137±2.07 in AngII group vs. 119.2±2.26 mmHg in AngII/ICG-001 group, n = 7-9, p<0.05) accompanied with a 34% (34 of 100) increase in 24-h urine volume and a 35% (35 of 100) increase in 24-h urinary Na+ excretion.

Following AngII treatment, urinary renin activity and renin content both exhibited a more than 10-fold increase which was completely blocked by ICG-001. AngII infusion selectively elevated α-ENaC protein abundance in the renal medulla but not in the renal cortex; the renal medullary upregulation of α-ENaC protein expression was attenuated by ICG-001. Similarly, renal cortical NCC and NKCC2 protein expression was elevated by AngII infusion; this upregulation of NCC and NKCC2 protein expression was again abolished by ICG-001. In contrast, NHE3 protein abundance remained constant. In cultured mpkCCD cells transfected with a β-catenin-driven luciferase construct, AngII treatment at 500 nM for 24 h induced a 10-fold increase in the reporter activity which was sensitive to ICG-001 (1 µM). In these cells, the AngII treatment induced amiloride-sensitive Na+ transport as assessed by epithelial volt-ohmmeter, which was completely blocked by ICG-001. Protein expression of α-ENaC exhibited the similar pattern of changes as ENaC activity whereas the expression of β- or γ-ENaC remained constant. ICG-001 was not associated with noticeable toxicity in vivo or in vitro. In summary, these results suggest that β-catenin signaling mediates AngII-induced hypertension at least in part through regulation of intrarenal renin response and the expression of Na+ transporters in the distal nephron.
In vivo, guanine moieties in DNA, RNA, guanine nucleotides or guanosine can undergo nitration (e.g., by peroxynitrite) or hydroxylation (e.g., by superoxide anion) on position 8 of the purine ring. Catabolism of these biomolecules leads to the in vivo production of a diverse group of 8-nitro, 8-amino and 8-hydroxy guanosine and guanine compounds. Since the renal effects of these compounds are entirely unknown, we examined in rats the effects of guanosine, guanine, 8-nitroguanosine, 8-nitroguanine, 8-hydroxyguanosine, 8-hydroxyguanine, 8-hydroxy-2-deoxyguanosine, 8-aminoguanosine and 8-aminoguanine (33 µmoles/kg/min; intravenous infusion for 115 minutes) on excretion of Na⁺, K⁺ and glucose. Guanosine, 8-nitroguanosine and 8-hydroxy-2-deoxyguanosine had minimal activity. Guanine, 8-nitroguanine, 8-hydroxyguanosine and 8-hydroxyguanine had moderate natriuretic activity (increased sodium excretion by 9.4-fold, 7.8-fold, 7.1-fold and 8.6-fold, respectively). In contrast, 8-aminoguanosine (n=6) and 8-aminoguanine (n=6) were highly efficacious and increased Na⁺ excretion by 26.6-fold (from 5.13 ± 2.16 to 136.44 ± 20.14 µmoles/30 min) and 17.2-fold (from 4.38 ± 2.00 to 75.28 ± 23.37 µmoles/30 min), respectively. 8-Aminoguanosine and 8-aminoguanine also increased glucose excretion by 12.1-fold (from 36.68 ± 11.22 to 445.11 ± 63.78 µg/30 min) and 12.2-fold (from 31.40 ± 16.70 to 382.68 ± 97.81 µg/30 min), respectively, and decreased K⁺ excretion by 69.1% (from 51.11 ± 11.37 to 15.78 ± 2.74 µmoles/30 min) and 71.0% (from 28.57 ± 6.62 to 8.28 ± 2.14 µmoles/30 min), respectively. Radiotelemetry studies demonstrated that 8-aminoguanosine (n=3) and 8-aminoguanine (n=3) in drinking water (5 mg/kg/day) suppressed deoxycorticosterone/salt-induced (DOCA/salt) hypertension. For example, in untreated DOCA/salt rats, MABP increased from 103 ± 4 to 184 ± 9 mm Hg after 65 days of DOCA/salt; whereas in 8-aminoguanosine-treated DOCA/salt rats, MABP increased from 98 ± 2 to 148 ± 9 mm Hg during the same time period. We conclude that 8-aminoguanosine and 8-aminoguanine are endogenous, potent and efficacious K⁺-sparing diuretics/natriuretics that may regulate renal function and blood pressure and may represent a new class of antihypertensive drugs.

E.K. Jackson: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; DK091190, HL069846, DK068575, HL109002, DK079307. D.G. Gillespie: None. Z. Mi: None.
We previously reported that neurogenic hypertension is associated with a reduction of Angiotensin Converting Enzyme 2 (ACE2) activity and an increase in A Disintegrin And Metalloprotease 17 (ADAM17) activity in the hypothalamus. Since ADAM17 is known to be expressed in multiple cell types and can be activated by various receptors, we tested the hypothesis that neuronal AT1a receptors (AT1aR) are necessary for ADAM17-mediated ACE2 shedding during neurogenic hypertension.

DOCA-salt treatment (DOCA 1mg/g body weight sc + 1% saline p.o./3 weeks) was given to male neuronal AT1aR knockdown mice (AT1aR floxed crossed with Nefh-cre mice, 14-16 week-old) and their non-transgenic (NT) littermates (n=8/group). Following DOCA-salt treatment, enzyme activity assays were performed in hypothalamus of both DOCA-salt-treated and sham mice. Unlike in NT mice, ADAM17 activity was not increased by DOCA-salt treatment in the hypothalamus of neuronal AT1aR knockdown mice (153.7 ±23.8 vs. 128.5 ±11.6 FU/min, P=0.22). To assess whether impaired ADAM17 activation resulted from down-regulation of neuronal AT1aR, or was due to an attenuated BP rise, wild-type mice were infused with norepinephrine (NE; 4 ug/kg/min/14 d, n=6/group). Contrary to DOCA-salt hypertension, ACE2 activity in the hypothalamus remained intact during NE-induced hypertension and there was no significant increase in ADAM17 activity (59.1 ±4.3 vs. 52.3 ±11.7 FU/min, P=0.29). Using FACS, neurons, astrocytes and non-neuronal/non-astrocyte cells were sorted from the hypothalamus. A significant increase in ADAM17 mRNA resulting from DOCA-salt treatment (+1.72 ±0.19 fold vs. sham, P<0.05) was observed only in the neuronal population of NT mice but not in neuronal AT1aR knockdown mice. ERK phosphorylation, NOX4 expression and ROS production, all of which are involved in both intra- and extracellular mechanisms of ADAM17 activation were then assessed using Western blotting and DHE-staining. All were significantly impaired in the hypothalamus of neuronal AT1aR knock-down mice, compared to NT, after DOCA-salt treatment.

Taken together, our data provide strong evidence that activation of neuronal AT1aR is responsible for ADAM17-mediated ACE2 shedding and the maintenance of neurogenic hypertension.

J. Xu: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA : 15POST25000010. S. Sriramula: None. D.A. Martin: None. E. Lazartigues: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH : HL093178.

Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee) 072

Brain Epithelial Sodium Channels on Organum Vasculosum of the Lamina Terminalis Neurons Mediate Sympathoexcitatory Pressor Responses and Neuronal Excitation by Hypertonic NaCl

Brian J. Kinsman, Kirsteen N. Browning, Sean D. Stocker, Penn State Coll of Med, Hershey, PA

High dietary salt intake raises cerebrospinal fluid (CSF) [Na+] in salt sensitive subjects to elevate sympathetic nerve activity (SNA), and arterial blood pressure (ABP). This occurs through excitation of NaCl-sensitive sites in the brain, including the organum vasculosum of the lamina terminalis (OVLT). Intriguingly, intracerebroventricular (ICV) pretreatment with benzamil (a non-voltage gated Na+ channel
blocker) also attenuates those systemic responses to central NaCl. Thus, I hypothesized that benzamil acts on NaCl-sensitive OVLT neurons to attenuate neuronal excitation and elevated SNA and ABP in response to hypertonic NaCl. To evaluate this hypothesis, lumbar SNA and ABP were measured in anesthetized adult rats in response to ICV infusion of 0.15, 0.5, and 1.0 M NaCl with and without prior OVLT microinjection of benzamil (5nmol per 20nL). ICV infusion of NaCl produced concentration-dependent increases in lumbar SNA (0.15M: 101±3%, 0.5M: 118±3%, 1.0M: 130±9% n=4 per group, P<0.05) and mean ABP (0.15M: 1±1mmHg; 0.5M: 5±1mmHg, 1.0M: 12±2mmHg). OVLT microinjection of benzamil significantly attenuated the increase in lumbar SNA (0.15M: 100±2%, 0.5M: 108±2%, 1.0M: 115±3% n=4 per group, P<0.05) and mean ABP (0.15M: 1±0mmHg; 0.5M: 2±1mmHg, 1.0M: 6±2mmHg). In a parallel set of experiments, in vitro whole-cell recordings of OVLT neurons in slices were performed to assess whether blockade of epithelial sodium channels (ENaC) attenuated NaCl-induced excitation. Bath application of +7.5mM NaCl increase action potential (AP) discharge of OVLT neurons (n=11) from 0.4±0.16 Hz to 1.09±0.26 Hz (P<0.05). Subsequent addition of an ENaC selective concentration of benzamil (0.5µM) reversed AP discharge (0.75±0.24 Hz, P<0.05). Isotonic 0.5µM benzamil did not significantly change NaCl-sensitive OVLT neuron (n=13) AP discharge from baseline (0.54±0.14 Hz to 0.60±0.16 Hz, P>0.05). Collectively, this data indicates that elevations in CSF NaCl concentrations excite OVLT neurons via ENaC to elevate SNA and ABP.

B.J. Kinsman: None. K.N. Browning: None. S.D. Stocker: None.

Funding: Yes
Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

Hindbrain Angiotensin Type-2 Receptors and Hypertension

Annette D de Kloet, Lei Wang, Jacob A Ludin, Helmut Hiller, Justin A Smith, Deborah A Scheuer, Univ of Florida, Gainesville, FL; Urlike M Steckelings, Univ of Southern Denmark, Odense, FL; Eric G Krause, Colin Sumners, Univ of Florida, Gainesville, FL

The role of the angiotensin type-2 receptor (AT2R) in the neural control of cardiovascular homeostasis and hypertension is not well-characterized. Using a BAC transgenic AT2R-enhanced green fluorescent protein (eGFP) reporter mouse, dense localization of AT2R to GABA neurons was found within the nucleus of the solitary tract (NTS) of the hindbrain, an area important for regulating baroreflex function and blood pressure. This localization was confirmed by RNAscope fluorescence in situ hybridization. Considering that GABA is pressor in the intermediate NTS (intNTS), and that its effects are enhanced in models of neurogenic hypertension such as SHR and deoxycorticosterone acetate (DOCA)-salt rats, we tested the hypothesis that AT2R on GABA neurons in the NTS constitute a counter-regulatory, blood pressure lowering mechanism. In support of this idea, we have demonstrated that: (i) Optogenetic stimulation of GABA neurons in the intNTS of normal mice elicited a significant increase in blood pressure; (ii) mRNA levels for both the GABA synthetic enzyme Gad1 and for the AT2R were significantly increased in the intNTS of DOCA-salt hypertensive mice; (iii) Intracerebroventricular (ICV) infusion of the AT2R agonist Compound 21 (C21; 7.5 ng/h) into normotensive or DOCA-salt hypertensive mice elicited a significant reduction of systolic blood pressure, an effect that was much larger in hypertensive (126.2 ± 5.0 v. 139.8 ± 3.2 mmHg; n = 14; p = 0.02) than
in normotensive (119.8 ± 2.6 v. 126.5 ± 2.5 mmHg; n = 16; p = 0.04) mice and was accompanied by decreased levels of the GABA synthetic enzymes Gad1 and Gad2 in the intNTS; (iv) Finally, the crucial involvement of GABA neurons in the blood pressure lowering effect of AT2R was proven by the lack of any effect of ICV C21 in DOCA-salt hypertensive mice containing a selective knockout of AT2R from GABA neurons. These novel data provide strong evidence for an anti-hypertensive action of AT2R within the intNTS, an effect that is exerted through decreases in GABA transmission.


Funding: No
Funding Component:

074

Epigenetic Regulation of Brain (Pro)renin Receptor by Benzamil-sensitive Sodium Channel in DOCA-salt Hypertension

Dane D Jensen, Yumei Feng, Univ of Nevada Sch of Med, Reno, NV

Increased expression of the brain (pro)renin receptor (PRR) contributes to the development of salt-sensitive hypertension (SSH). Yet, the mechanisms that drive this increase in PRR expression are not known. We previously reported an increase in histone 3 lysine 4 trimethylation (H3K4me3), an epigenetic marker for gene activation, on the PRR promoter in SSH mice. We hypothesized that the increase in brain PRR expression is driven by epigenetic modifications linked to high-salt intake. To test our hypothesis, C57Bl/6J mice were treated with Sham (sham pellet + tap water for drinking) or DOCA-salt (50mg DOCA pellet + 0.9% saline) with chronic intracerebroventricular (ICV) infusion of the epithelial sodium channel (ENaC) blocker Benzamil (2.64μg/day, 0.5μl/h) or aCSF for 3 days. Hypothalamic tissues were used to examine the chromatin modifiers activity (ng/h/mg of protein) responsible for the methylation or demethylation of H3K4 including the histone methyltransferase (HMT) and histone demethylase (HDM). Water intake and PRR mRNA level were monitored. In mice receiving ICV aCSF, DOCA-Salt treatment increased the activity of H3K4 HMT (18.5 ± 1.2 vs. 6.6 ± 0.3, P<0.001) and HDM (0.55 ± 0.03 vs. 0.37 ± 0.05, P<0.05) compared to Sham respectively. HMT activity was blunted by ICV infusion of Benzamil (14.2 ± 0.5, P<0.01) compared to ICV infusion of aCSF following 3 days of DOCA-salt treatment. Interestingly, HDM activity was further elevated with the treatment of ICV Benzamil compared to ICV infusion of aCSF (0.84 ± 0.03, P<0.01). ICV infusion of Benzamil also reduced the PRR mRNA levels in the hypothalamus of mice treated with DOCA-salt from (1.43 ± 0.09, P<0.05) to (1.07 ± 0.02 P>0.05) relative to Sham (1.00). In addition, the saline intake amount (ml/day) was lower (P<0.05) in mice receiving ICV infusion of Benzamil (17 ± 3) compared to ICV aCSF (32 ± 9) following DOCA-salt treatment. In summary, ICV infusion of Benzamil attenuates the elevation in HMT activity, water and sodium intake, and the PRR mRNA levels, while increasing HDM activity in DOCA-salt treated mice. We conclude the Benzamil-sensitive sodium channel, possibly ENaC, may be a key channel regulating the epigenetic modifications governing the increased expression of brain PRR in SSH.
Impaired Hypothalamic PVN G-alpha i2 Signal Transduction Attenuates Endogenous Sympathoinhibitory PVN GABAergic Tone to Evoke the Development of Salt-sensitive Hypertension

Richard D Wainford, Joon Shim, Boston Univ, Boston, MA

Aim: We hypothesize hypothalamic PVN Gαi2 proteins, which are up regulated during high salt intake, facilitate sympathoinhibition and blood pressure regulation via mediating GABA_a receptor signal transduction to maintain salt-resistance in the Sprague Dawley (SD) rat.

Methods: Groups of male salt-resistant SD rats received a bilateral PVN infusion of a scrambled (SCR) or Gαi2 oligodeoxynucleotide (ODN-300ng/side/day) and a normal 0.6% (NS) or high 4% NaCl (HS) diet for 7-days. On day-7 24h Na+ balance was assessed - in sub-groups MAP, plasma norepinephrine (NE) content, PVN GAD67 [marker of GABAergic expression] and vGLUT2 [marker of glutamatergic expression] immunoreactivity, and the cardiovascular responses to bilateral administration of the GABA_a receptor antagonist (CGP52432; 3nmol/side) and the ionotropic glutamate receptor antagonist (kynurenate; 1.4 μg/side) was assessed (N=4/gp/study).

Results: In control SCR ODN PVN infused SD rats HS intake suppressed plasma NE (plasma NE [nmol/L] SCR NS 76±7 vs HS 43±6, P<0.05) and evoked increased GAD67, but not vGLUT2, immunoreactivity (Medial Parvocellular (MP) GAD67 [relative fold change (normalized to DAPI) 8.4±1.1] in all PVN parvocellular sub-regions without impacting MAP or sodium balance. Following ODN-mediated PVN Gαi2 down-regulation HS intake caused salt-sensitive hypertension (MAP [mmHg] Gαi2 ODN NS 128±3, HS 148±4, P<0.05), failed to increase GAD67 expression and increased PVN vGLUT2 immunoreactivity in multiple PVN parvocellular sub-regions (MP vGLUT2 [relative fold change (normalized to DAPI) 3.2±0.8]). In separate groups of rats bilateral PVN GABA_a antagonist increased MAP in control SCR but not Gαi2 ODN infused rats maintained on a HS intake (peak ΔMAP [mmHg] SCR ODN HS +17±3, Gαi2 ODN HS +2±2). In contrast, bilateral PVN glutamate antagonism did not alter MAP in control SCR ODN infused rats but decreased MAP in hypertensive Gαi2 ODN infused SD rats on a HS intake (peak ΔMAP [mmHg] SCR HS +3±3, Gαi2 ODN HS -13±2).

Conclusion: These data suggest impaired Gαi2 signaling attenuates sympathoinhibitory PVN GPCR-mediated GABAergic tone (i.e., modulates GPCR coupled GABA_a responses) and enhances sympathoexcitatory glutamatergic tone to evoke salt-sensitive hypertension in the SD rat.

R.D. Wainford: B. Research Grant (includes principal investigator, collaborator, consultant and pending grants as well as grants already received); Modest; B. Research Grant (includes principal investigator, collaborator, consultant and pending grants as well as grants already received); Significant; NIH R01 HL107330, K02 HL112718, Novartis Investigator Initiated Support. D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Japanese Hypertension Summit 2016. E. Honoraria; Modest; Emory University
Brain-targeted Bradykinin B1 Receptor Blockade Prevents DOCA-salt Hypertension by Oxidative Stress and ERK1/2 Phosphorylation Mediated Signaling Mechanisms

Srinivas Sriramula, Eric Lazartigues, LSU Health Sciences Ctr, New Orleans, LA

DOCA-salt hypertension is associated with increased expression of the pro-inflammatory bradykinin B1 receptor (B1R) in the brain. We previously reported that DOCA-salt hypertension is attenuated in global B1R knockout mice. In this study, we further examined the role of B1R within the central nervous system, on hypertension and cardiac hypertrophy, and explored the contribution of downstream signaling mechanisms. DOCA-salt treatment (1 mg/g body weight DOCA, 1% saline in drinking water, 3 weeks) of C57Bl6/J male mice produced a significant rise in mean arterial pressure (MAP; telemetry) in these animals (145 ±3 mmHg, n=8, p<0.01). Intracerebroventricular (ICV) infusion of R715 (70 µg/kg/day, 3 weeks), a specific B1R antagonist, attenuated the DOCA-salt-induced hypertension (125 ±3 mmHg, n=8, p<0.01). Moreover, DOCA-salt treatment resulted in cardiac hypertrophy in C57 compared to sham-treated mice (heart weight/tibia length; 9.2 ±0.3 vs. 7.2 ±0.2 mg/mm), which was prevented by B1R blockade with R715 (7.4 ±0.3 mg/mm; P<0.01 vs. DOCA). DOCA-salt-treated mice had increased inflammation (qRT-PCR), as shown by elevated gene expression of TNF, IL-1β and IL-6 (3-, 8- and 5-fold, respectively, n=9, p<0.01 vs. sham) in the hypothalamic paraventricular nucleus (PVN), which was attenuated in R715-treated mice. C57 mice treated with DOCA-salt showed increased oxidative stress, as indicated by increased gene expression of Nox-2 and iNOS (5- and 15-fold increase, respectively, n=4, p<0.01 vs. sham) in the PVN which was prevented by central B1R blockade with R715 (1.9- and 4.4-fold increase, respectively, n=4, p<0.01 vs. DOCA). DOCA-salt-induced increase in PVN Nox-2 protein expression was also prevented by central B1R blockade (2.2 vs.1.1-fold change, p<0.01). Furthermore, phosphorylation of ERK1/2 was increased in the PVN of DOCA-salt-treated mice (66 ±3 % increase, n=4, p<0.05 vs. sham), which was blunted in R715 treated mice. Taken together, these data provide evidence that blockade of brain B1R attenuates DOCA-salt-induced hypertensive and hypertrophic effects via oxidative stress and ERK1/2 phosphorylation-mediated signaling mechanisms. (Funding: AHA 15SDG25720021 (SS), NIH/NHLBI (HL093178) (EL), and P30GM106392).

S. Sriramula: None. E. Lazartigues: None.

Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

Role of Suppressor of Cytokines Signaling 3 (SOCS3) in POMC Neurons in Regulating Metabolic and Cardiovascular Functions in Dietary-Induced Obesity

Zhen Wang, Jussara do Carmo, Univ of Mississippi Medical Ctr, Jackson, MS; Alexandre da Silva, Ctr Universitário Barão de Mauá, Ribeirão Preto, Brazil; Nicola Aberdein, John Hall, Univ of Mississippi Medical Ctr, Jackson, MS
Suppressor of cytokine signaling 3 (SOCS3), a negative regulator of leptin signaling, may contribute to the development of obesity-induced leptin resistance. Previously, we showed that activation of proopiomelanocortin (POMC) neurons mediates the chronic effects of leptin on blood pressure (BP) and glucose regulation. However, the role of SOCS3 in POMC neurons in regulating metabolic and cardiovascular functions in obesity is still unclear. To address this question, we used male and female mice with SOCS3 deleted only in POMC neurons (SOCS3^{flox/flox}-POMC/cre) and flox control (SOCS3^{flox/flox}) mice. After weaning, mice were fed normal chow until 20 wks of age; then glucose tolerance tests (GTT) were performed and telemetry probes were implanted to measure mean arterial pressure (MAP) 24-hrs/day. We found that at 23 wks of age, both male and female SOCS3^{flox/flox}-POMC/cre mice weighed less than control mice (32±1 vs 37±2 g in male and 25±1 vs 27±1 g in female, n=9-11, *p*<0.05), but had similar daily food intake (3.5±0.2 g in male and 3.3±0.1 g in female) and MAP (114±1 vs 115±2 mmHg). Only male SOCS3^{flox/flox}-POMC/cre mice exhibited improved glucose tolerance (AUC: 1059±52 vs 1283±54 mg/dL x 120 min, n=7-10, *p*<0.05) compared to controls. From 23 wks, mice were switched to a high fat diet (45%, HFD) for 6 wks. After HFD feeding, both male and female SOCS3^{flox/flox}-POMC/cre mice had slightly reduced food intake (3.2±0.1 vs 3.5±0.2 g in male and 2.7±0.1 vs 3.0±0.2 g in female) and a trend toward lower body weight gain (10±1 vs 12±1 g in male and 4±1 vs 6±1 g in female), although the differences were not significant compared to controls. However, male and female SOCS3^{flox/flox}-POMC/cre mice fed a HFD had significantly greater MAP increase (7±1 vs 1±1 mmHg, n=13-16, *p*<0.05) and an enhanced BP response to acute air-jet stress (AUC: 109±9 vs 74±12 mmHg x 5min, n=8-13, *p*<0.05). After HFD, glucose tolerance was impaired in all groups of mice compared to the baseline at 20 wks, but there were no differences between SOCS3^{flox/flox}-POMC/cre and controls. These results suggest that deletion of SOCS3 in POMC neurons amplifies the BP response to a HFD and to acute stress, but has minimal effects on metabolic functions to HFD. (NHLBI PO1HL51971, NIGMS P20GM104357, AHA 14POST18160019)


Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

078

**Postnatal Treatment with Metyrapone Attenuates the Effects of Early Life Stress (ELS) on Diet-induced Obesity in Female Rats**

Margaret O Murphy, Dianne M Cohn, Analia S Loria, Univ of Kentucky, Lexington, KY

Clinical studies have shown a positive correlation between ELS and the development of cardiometabolic disease, particularly affecting women. We previously reported that male rats exposed to Maternal Separation (MatSep), a model of ELS in rodents, do not develop exaggerated diet-induced obesity. Thus, this study tested the hypothesis that MatSep exacerbates the response to an obesogenic diet in female rats. Also, we tested whether the postnatal treatment with metyrapone (MTP), a corticosterone synthase inhibitor, would attenuate this phenotype. MatSep was performed in WKY offspring during 3 hours/day from postnatal days 2-14. Non-disturbed littermates were used as controls. Female rat offspring were untreated or treated with MTP (50 mg/kg, i.p.), 30 minutes prior the daily separation. Upon weaning, rats were
placed on regular chow (ND, 18% kcal fat) or HFD (60% kcal fat) for 12 weeks. Despite no differences in food intake (metabolism cages) and blood pressure (DSI radiotelemetry) MatSep exaggerated body weight gain and fat pad weights (p<0.05) in response to HFD. Also, MatSep increased plasma corticosterone (189±48 vs. 79±18 pg/ml, p<0.05) and leptin (2.1±0.4 vs. 1.5±0.4 ng/ml, p<0.05) levels compared to control while insulin and adiponectin levels were similar between groups. Oral glucose tolerance test was impaired in MatSep rats showing a greater AUC compared to control rats (p<0.05). Importantly, MTP-treated female MatSep rats showed significantly attenuated diet-induced obesity, glucose intolerance, plasma corticosterone and leptin levels. Histological analysis revealed that MTP treatment ameliorated the adipocyte size in visceral fat from MatSep rats as well (p<0.05). Gene expression in liver indicated that glucose 6 phosphatase, but not other gluconeogenic enzymes, were increased in obese MatSep rats, whereas the MTP treatment abrogated this effect. The visceral fat gene expression showed lower levels of insulin receptor in MatSep rats, but no differences in other genes related to the glucose disposal. Overall, these data reveal that female MatSep rats display a greater susceptibility for the synergistic effect between obesogenic diet and ELS compared to male rats, and this effect may be linked to early life exposure to stress hormones.

M.O. Murphy: None. D.M. Cohn: None. A.S. Loria: None.

Funding: No

Funding Component:

079

Hmox1 Activation Reprograms White Fat to Beige Adipose Tissue Through Recruitment of Cyp2C44-derived EET, pAMPK-PGC1α That Enhances Mitochondrial Mfn2 and Opa1


Introduction: Hmox1+/− is critical in the regulation of insulin sensitivity and mitochondrial bioenergetics by regulating cellular heme-derived CO and bilirubin levels (Hosick and Stec 2015). We have demonstrated that high fat (HF) diets decreased HO-1 and PGC-1α. We examined the role of HO-1 on white and brown-fat like and energy expenditure genes. Methods: C57/B16 female mice, 5 wks old, were fed a HF diet for 24 wks. At 12 wks, when all mice had established pre-diabetic stage, cobalt protoporphyrin, (CoPP) was administered, i.p. (0.3 mg /100, BW), for the last 8 wks. Blood and fat tissues were collected, Cyp-2C44-derived EET, AMPK, HO-1, insulin receptors, and mito-fusion protein markers were determined in the three groups of mice: A) Control, B) HF, C) HF-CoPP. We generated adipoq-HO-1 knockout mouse model and mRNA and protein signaling, adiposity, oxygen consumption were determined and compared to adipocyte culture depressed in HO. Result: CoPP increased HO-1 expression by 20-fold (p<0.02), EETs (p<0.05), PGC-1α (p<0.05), pAMPK (p<0.05) and Mfn1/2 (p<0.05), Opa1 and OXPHOS (p<0.05), oxygen consumption (Vo2), but decreased fasting glucose levels. These was associated with a decreases in VAT, SAT (p<0.02). Adipoq-HO-1−/− female mice exhibit marked decrease in pAMPK, Cyp2C44, PGC1α, Mfn1/2, Opa1, UCP1 and SIRT1 but increased adipogenic markers including Mest/Peg1 in adipose tissues.

Conclusion: Overexpression of HO-1-derived CO
and bilirubin enhances UCP1, Cyp2C44, pAMPK-PGC1α to turn on a robust program of energy expenditure genes and reprogrammed white (VAT, SAT) to beige adipose and brown-like adipocytes. Deletion of HO-1 or suppression of HO-1 led to metabolic dysfunction, hyperglycemia as in white fat to exhibit significant low energy expenditure, OXPHOS and VO₂. Mechanistically, absence of HO-1 decreased AMPK signaling; thereby disrupting EET-PGC-1α expression and mitochondrial oxidative metabolism. These findings offer new insight for the development of obesity treatment strategies through restoration of HO-1 and adipocyte function.


Funding: No

Funding Component:

080

mTORC1 as a Novel Regulator of Vascular Endothelial Function in Obese Mice and Humans

John J Reho, Deng-Fu Guo, Andrew Olson, Lauren Wegman-Points, Isaac Samuel, Peter Nau, Jessica Smith, Gary L Pierce, Kamal Rahmouni, Univ of Iowa, Iowa City, IA

Obesity-induced hypertension is associated with vascular endothelial dysfunction. Recently, our laboratory has demonstrated a critical role of the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway in cardiovascular regulation. Here, we tested the hypothesis that dysregulation of mTORC1 signaling is involved in the endothelial dysfunction associated with obesity in mice and humans. We found that diet-induced obese (DIO) mice that display vascular endothelial dysfunction as compared to lean controls have increased mTORC1 signaling in aortic lysates indicated by the elevated (p<0.05) phosphorylated levels of mTOR and its downstream signaling targets S6-kinase and the ribosomal S6 protein measured by Western blot. Increased vascular mTORC1 signaling in DIO mice was associated with increased aortic NOX2 mRNA expression (2.0±0.2 vs. 1.0±0.3AU in lean controls; p<0.05). Isolated abdominal subcutaneous adipose arterioles from non-diabetic obese (BMI ≥30 kg/m²; n=4; age 51±6 yrs; BMI 54±3 kg/m²) humans exhibited a strong trend towards increased phosphorylated S6 protein compared to normal-weight (BMI <30kg/m²; n=3; age 44±15 yrs; BMI 26±1 kg/m²) individuals (5.0±1.9 vs 0.8±0.4AU; p=0.12), suggesting increased vascular mTORC1 signaling in human obesity. Next, we used an adenoviral construct of a constitutively active (CA) S6-kinase (Ad-CAS6K) to enhance mTORC1 signaling. In mouse endothelial cells, Ad-CAS6K increased mRNA expression of oxidative stress (NOX1and NOX2) and inflammatory markers (IL-1β) and decreased endothelial NOS expression (p<0.05). Transfection of aortic rings with the Ad-CAS6K resulted in impairment in acetylcholine-induced relaxation (Max. relaxation: 67± 5 vs. 81 ±3%; p<0.05) without altering the relaxation evoked by sodium nitroprusside (Max. relaxation: 90±1% vs. 90±2%) recapitulating the vascular phenotype in obese mice. Taken together, our data demonstrate a novel role of the mTORC1 signaling pathway in the regulation of vascular endothelial function. Our data also implicate dysregulation of the endothelial mTORC1 signaling pathway in the endothelial dysfunction associated with obesity.


Funding: Yes
Alterations in cardiac baroreflex sensitivity (BRS) and 24-hr blood pressure variability (24-hr BPV) are independent predictors of increased cardiovascular disease (CVD) risk, and occur in individuals with obesity. Obese humans are also likely to have a higher large elastic artery stiffness compared with normal-weight individuals. While an increase in stiffness of carotid and aortic arteries, the anatomical sites where baroreceptors reside, may likely be responsible in part for the decline in cardiac BRS with advancing age in adults, it remains unclear whether 1) elevated carotid and aortic stiffness are also directly associated with obesity-associated reductions in cardiac BRS in young/middle-aged individuals, and 2) if reduced BRS with obesity is associated with elevated 24hr BPV. We tested the hypothesis that lower BRS would be associated with higher carotid and aortic stiffness and 24hr BPV in young and middle-aged individuals with obesity. In a cross-sectional design, 22 normal-weight (body mass index, BMI 24.5 ± 0.6 kg/m²; age 35±2 yrs; 8M/14F) and 22 obese (BMI 34.2 ± 1.1 kg/m²; age 39 ± 2 yrs; 8M/14F) individuals underwent measures of spontaneous cardiac BRS (sequence technique), carotid artery β-stiffness (carotid tonometry and B-mode ultrasound of common carotid artery), aortic stiffness (carotid-femoral pulse wave velocity, CFPWV), and 24-hr-systolic BPV (24 hr ambulatory BP monitoring). A significant relation between cardiac BRS and 24-hr systolic BPV (r=-0.42, P<0.01) was corroborated by lower cardiac BRS (11.7±1.2 vs. 16.8±1.7 ms/mmHg, P<0.05) and higher 24-hr BPV (12.4±0.6 vs. 10.1±0.4 mmHg SD, P<0.05) among obese compared with normal-weight subjects. In contrast, carotid β-stiffness (7.8±0.6 vs. 6.9±0.4 U, P>0.05) and CFPWV (745±71 vs. 611±19 cm/s, P=0.07) were not significantly different between groups despite greater average 24-hr systolic BP in the obese vs. normal weight subjects (127±2 vs. 118±1 mmHg, P<0.05). These preliminary data suggest that an increase in carotid artery and aortic stiffness may not precede the decline in cardiac BRS and increase in 24hr BPV in young and middle-aged obese individuals, suggesting non-arterial stiffness related mechanisms for obesity-related reductions in cardiac BRS.


Funding: No
Activation of the renin-angiotensin system, and in particular angiotensin (Ang) II, is observed in obese patients and closely correlates with insulin resistance. Obesity is also associated with deficiency of Ang-(1-7), a vasodilatory peptide that mitigates Ang II actions. Accumulating evidence suggests that Ang-(1-7) has direct positive metabolic effects including reducing adiposity and reversing whole-body glucose intolerance and insulin resistance in animal models of cardiometabolic syndrome. In this study, we tested the hypothesis that chronic Ang-(1-7) administration would prevent high fat diet-induced obesity in mice, and determined potential mechanisms underlying this effect. To test this hypothesis, adult male C57BL/6J mice received a 12-week systemic infusion of Ang-(1-7) (400 ng/kg/min; n=7) or saline (n=6) via subcutaneous osmotic mini-pumps. Immediately following mini-pump implantation, mice were placed on a 60% high fat diet, with body mass measured weekly. Body composition (mq10 nuclear magnetic resonance analyzer), food and water intake, and energy expenditure (indirect calorimetry) were measured during the last week of treatment. Ang-(1-7) attenuated high fat diet-induced weight gain [39.7±1.3 Ang-(1-7) vs. 43.9±0.7 g saline at 12 weeks; p=0.023] and adiposity [32±1 Ang-(1-7) vs. 29±1% saline; p=0.050]. The reduced gain in body weight following Ang-(1-7) was associated with increased average energy expenditure [0.55±0.02 Ang-(1-7) vs. 0.42±0.06 kcal/hour saline, p=0.038] and oxygen consumption [1.84±0.07 Ang-(1-7) vs. 1.39±0.23 ml/min saline, p=0.049] during the dark cycle, with no effect on locomotor activity. A similar trend for Ang-(1-7) to increase energy expenditure and oxygen consumption was observed during the light cycle. There were no significant differences in food or water intake following chronic Ang-(1-7) versus saline infusion. These findings suggest that targeting of Ang-(1-7) may be a novel strategy to prevent the development of obesity by enhancing energy expenditure, and further highlight the importance of the renin-angiotensin system in metabolic regulation.

A.C. Arnold: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH HL122507. C. Finney: None. I. Biaggioni: None.

Funding: No
Funding Component:

083

γ/δ T Cells Play a Role in Development of Hypertension


Objective: Both innate antigen-presenting cells and the adaptive immune system have been shown to play a role in the development of hypertension. Nevertheless, the T cell subsets involved in the pathophysiology of hypertension remains unclear. There is a small subset of “innate-like” T cells expressing the γ/δ T cell receptor (TCR) rather than the α/β TCR that could play a role bridging the innate and adaptive immune systems. We previously
observed that angiotensin (Ang) II caused an increase in number and activation of γ/δ T cells and Ang II-induced systolic blood pressure (SBP) rise and vascular injury were blunted in Tcrδ−/− mice, which are devoid of γ/δ T cells. In order to further characterize the role of γ/δ T cells in hypertension, we determined whether Ang II-effects would be blunted by antibody-induced depletion of γ/δ T cells. In addition, we tested whether SBP in human could be predicted by combining the expression of genes encoding TCRGC (TCR gamma constant region) and pro-inflammatory markers in peripheral blood mononuclear cells (PBMC).

**Method and Results:** Thirteen to 15-week old male C57BL/6 wild-type (WT) mice were infused with Ang II (490 ng/kg/min, SC) for 14 days and injected IP with anti-γ/δ TCR or control isotype antibodies 1 day before and at day 6 of Ang II infusion. Depletion of γ/δ T cells decreased SBP (147±2 vs 167±3 mm Hg, P<0.05) and restored mesenteric artery relaxation responses to acetylcholine (Emax: 90±4 vs 62±8%, P<0.05) compared to isotype antibody-treated mice. Using the SBP data and the PBMC gene expression profile (GSE12288) of 222 human subjects, we predicted with a supervised machine learning approach SBP by combining the gene expression of TCRGC and pro-inflammatory makers including interleukin-17A, interferon-γ and their receptors (R=0.23, P<0.001).

**Conclusion:** Antibody-induced depletion further demonstrates the role of γ/δ T cells in Ang II-induced SBP elevation and vascular injury. Prediction of SBP using PBMC gene expression of γ/δ T cells and pro-inflammatory markers suggests that γ/δ T cells contribute to the development of human hypertension.


**Sodium Butyrate and Retinoic Acid Attenuate Renal Inflammation and Fibrosis in Npr1 Gene-targeted Haplotype Mice: Role of NF-κB Signaling**

Prerna Kumar, Ramu Periyasamy, Venkateswara Gogulamudi, Kailash N Pandey, Tulane Univ Health Sciences Ctr, Sch of Med, New Orleans, LA

The objective of the present study was to determine the effect of a hybrid drug of sodium butyrate (NaBu), a histone deacetylase (HDAC) inhibitor and all-trans retinoic acid (ATRA) on the attenuation of renal inflammation and fibrosis in Npr1 gene-disrupted mutant mice. Adult (18-20 week old) male Npr1 gene-disrupted heterozygous (1-copy; Npr1+/−) wild-type (2-copy; Npr1+/+), and gene-duplicated (3-copy; Npr1++/+) mice were treated with ATRA-NaBu hybrid drug (1.0 mg/kg/day) by intraperitoneal injections for 2-weeks. A significant decrease in systolic blood pressure was observed in ATRA-NaBu-treated haplotype Npr1+/− mice compared with untreated controls (treated, 113.3 ± 1.5 vs. control, 130.4 ± 1.9; p < 0.01). After treatment with ATRA-NaBu, a marked reduction in tubulo-interstitial fibrosis (50%, p < 0.001) and decreased renal collagen type I alpha 2 expression by 55% (treated, 25.9 ± 1.2 vs. control, 57.2 ± 2.9, p < 0.001) was observed in Npr1+/− mice. Treatment with ATRA-NaBu also increased creatinine clearance (ml/24 h) in Npr1+/− mice (245.7 ± 24.3 vs. control, 83.8 ± 3.9). Similarly, higher urinary albumin to creatinine ratio was detected in Npr1+/− mice (0.84 ± 0.03) vs. control (0.35 ± 0.03; p < 0.01) and a complete reversal was observed in drug-treated Npr1+/− mice (0.39 ± 0.05). Significant decreases were observed in renal (pg/mg...
protein) tumor necrosis factor-alpha (TNF-α) (4.1 ± 0.7 vs. control, 27.2 ± 2.6; p < 0.01) and interleukin (IL)-6 (11.8 ± 0.8 vs. control, 59.9 ± 3.6; p < 0.01) in ATRA-NaBu-treated Npr1+/− mice. Western blot analyses showed significant reduction in renal TNF-α and IL-6 protein expression by 54%, (p < 0.001) and 61%, (p < 0.01), respectively, in ATRA-NaBu-treated Npr1+/− mice. There was 49% increase in renal NF-κB (p65) DNA binding activity in Npr1+/− mice and 51% lower activity in Npr1+/+++ mice compared with wild-type mice. Western blot analysis revealed distinctly higher levels of renal NF-κB (p65) protein expression in Npr1+/− mice and reduced levels in Npr1+/+++ mice compared with wild-type controls. The present results provide direct evidence that ATRA-NaBu hybrid drug acts as a potent anti-inflammatory agent, which will have important implications in the pathophysiology of renal injury and hypertension.

P. Kumar: None. R. Periyasamy: None. V. Gogulamudi: None. K.N. Pandey: None.

Funding: No

The Role of CD70 in Vascular Function

Hana A. Itani, Arvind Pandey, Jonathan D. Brown, David G Harrison, Vanderbilt Univ, Nashville, TN

We have recently shown that CD70 is important in formation of effector memory T cells and that these cells seem to enhance the hypertensive response and end organ damage caused by repeated hypertensive stimuli. CD70 has traditionally been identified on antigen presenting cells and is thought to interact with CD27 on T cells, promoting proliferation and memory cell formation. The precise cell types that express CD70 and the role of CD70 on non-immune cells has not previously been investigated. In our prior study, we showed that hypertension is associated with a marked increase in CD70 surface expression on dendritic cells (DCs) and macrophages. To examine the specific effect of DC CD70, we performed adoptive transfer of DCs from hypertensive WT mice to normotensive CD70−/− mice and from hypertensive CD70−/− mice to normotensive WT mice. The recipient mice were treated with a normally subpressor dose of ang II (140 ng/kg/min). Interestingly the CD70−/− recipients were protected from the development of hypertension, despite receiving WT DCs from hypertensive donors. In contrast, the WT recipients that received CD70−/− DCs developed modest hypertension, indicating that non-DC sources of CD70 likely contribute to the hypertensive phenotype. Studies of mesenteric vascular reactivity showed that CD70−/− mice have markedly impaired endothelium-dependent vasodilatation to acetylcholine compared to WT mice (43±10 vs 25 ± 3 %) at baseline. In contrast, there were no differences in relaxation responses to sodium nitroprusside. In additional experiments, we showed that human umbilical vein endothelial cells express CD70 mRNA and that this is increased by > 30 fold by laminar shear stress (15 dynes/cm²) compared to oscillatory shear. Finally, using immunohistochemistry, we identified CD70 protein localized to resistance vessels of the kidney of ang II-treated mice. These data identify a new role of CD70 in modulating vascular function independent of its role on antigen presenting cells in memory T cell formation.

H.A. Itani: None. A. Pandey: None. J.D. Brown: None. D.G. Harrison: None.

Funding: No
The renin-angiotensin system (RAS) plays a key role in the modulation of multiple functions in many organs. It has been demonstrated that the protective axis Ang-(1-7)/ACE2/Mas exerts anti-inflammatory effects in different disorders. Along this line, we have shown that Mas receptor deficiency exacerbates lipopolysaccharide (LPS)-induced cerebral and systemic inflammation in mice. In this study, we investigate the effects of LPS-induced shock in a mouse model for global increases in Mas receptor expression (UB8 mice). Mice were injected intraperitoneally (i.p.) with LPS, and experiments were performed 1h (RT-qPCR and ELISA) and 6 h (Echocardiography, NAG activity, and Lung histological analysis) after injection (except for the survival curve experiment). Cytokine gene expression was significantly attenuated in the left ventricle (LV) of UB8 vs. FVBN mice (p<0.05) the 1 hour after LPS (5mg/kg) administration: IL6 (119.7 ± 26.9 vs.199.6 ± 31.9 a.u), TNFα (59.3 ± 5.8 vs.89.2 ± 5.9 a.u), IL1β(56.6 ± 11,5 vs.122.8 ± 20.4 a.u) and IL10 (33.8 ± 3.5 vs.72.6 ± 5.8 a.u). Similar results were observed in serum protein levels: IL6 (6,651 ± 946.4 vs.3,203 ± 904.1 pg/ml), TNFα (925.5 ± 56.6 vs. 1,380 ± 114.1 pg/ml), IL1β (2,844 ± 462.2 vs. 5,876 ± 841.5 pg/ml) and IL10 (8.8 ± 6.4 vs.230.6 ± 54.4 pg/ml). In FVBN mice, LPS (15 mg/kg) significantly decreased stroke volume (before: 31.92 ± 2.44 after LPS: 17.50 ± 1.61 ul), cardiac output (before: 30.92 ± 2.35 after LPS: 16.83 ± 1.56 ml/min) but in the LPS-treated UB8 mice, no changes were observed in cardiac function. Additionally, Increases in NAG activity in the LV and lung and in Alveolar Wall Thickening (AWT) were seen only in FVBN mice after LPS administration (LV NAG FVBN sham vs. FVBN LPS: 0.051 ± 0.008 vs. 0.096 ± 0.004 U/L and lung: 0.030 ± 0.034 vs. 0.673 ± 0.093; AWT FVBN sham: 0.794 ± 0.160 vs FVBN LPS 1.781 ± 0.118 μm2). Finally, we found a significant increase in survival of UB8 mice (60.32 %) vs. FVBN (48.3%) after LPS administration (30mg/kg). Taken together, these results indicate that global increase in receptor Mas expression in mice exerts protective actions in heart and lung against LPS challenge, highlighting the therapeutic potential of Mas axis during inflammatory processes.

D. Motta-Santos: None. O. Oliveira-Lima: None. G. Magalhães: None. C. Rocha-Resende: None. S. Scalzo: None. M. Barrouim Melo: None. J. Carvalho-Tavares: None. UFMG, Belo Horizonte, Brazil; Natalia Alenina, Michael Bader, MDC, Berlin-Buch, Germany; Maria José Campagnole-Santos: None. Silvia Cristina Fonseca Guatimosim: None. Robson Augusto Souza dos Santos, UFMG, Belo Horizonte, Brazil

Funding: No
inflammation associated with endothelial dysfunction. Interestingly, recent studies have identified mitochondrial adaptation and/or dysfunction as components to hypertensive vascular dysfunction. While mitochondria are indispensable to maintain cellular metabolism, they also participate in adaptive and maladaptive cell/tissue responses via several retrograde signaling pathways. DRP1 plays a major role in mitochondrial quality control. However, whether DRP1 is involved in mitochondrial dysfunction and endothelial inflammation during development of HTN remains unknown. In the present study, we tested the hypothesis that inflammatory stimuli, through DRP1-dependent mitochondrial alteration, enhance endothelial inflammation.

In cultured rat aortic endothelial cells (RAECs), TNFα (10 μg/mL) transiently induced mitochondrial fission maximally at 3h which was inhibited using a mitochondrial fission inhibitor, Mdivi1 (10 μM) (0.16±0.04 vs 0.10±0.02 mitochondria fragmentation count with MitoTracker, p<.01). TNFα and FCCP (a fission agonist, 10 μM) increased THP-1 monocyte adhesion to RAECs, which was also inhibited with Mdivi1 (256±17 vs 139±16 for TNFα, 238±30 vs 156±14 for FCCP, attached cells per field scanned, p<.01). Likewise, mdivi1 and adenoviruses encoding siRNA for DRP1 or dominant-negative K38A DRP1 (50 moi) attenuated TNFα-induced VCAM-1 induction in RAECs. TNFα increased aerobic respiration, which was prevented by mdivi1 or ER stress inhibitor PBA (10 mM). Inhibition of ER stress, glycolysis or mitochondrial respiration using PBA, 2-DG (1 mg/mL) or oligomycin (1 μM) prevented VCAM-1 induction. However, suppression of TNFα-induced mitochondrial ROS production by mito-Tempo (25 nM) was unable to prevent VCAM-1 induction. In C57BL6 mice receiving AngII (1000 ng/kg/min, 2 weeks) infusion, treatment with Mdivi-1 (25 mg/kg ip every other day) or PBA (1g/kg/day) prevented vascular VCAM-1 induction. In conclusion, our data suggests a critical role for ER stress and subsequent functional and structural remodeling of mitochondria induced by DRP1 in mediating endothelial inflammatory activation in HTN.


Funding: Yes
Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

Chronic Efferent Vagal Stimulation Enhances Norepinephrine-mediated Inhibition of Splenic Pro-inflammatory Cytokine Release in Lupus Hypertension

Grace S Pham, Amber S Fairley, Keisa W Mathis, UNTHSC, Fort Worth, TX

Hypertension is prevalent in the autoimmune disease systemic lupus erythematosus (SLE), occurring with alarming frequency in reproductive-age women. Recent studies implicate the adaptive immune system in the development and maintenance of hypertension, and neuroimmune pathways may regulate this source of inflammation. One example is the cholinergic anti-inflammatory pathway (CAP), an endogenous nerve-to-spleen mechanism that regulates splenic pro-inflammatory cytokine release. We hypothesized that this pathway is impaired in SLE and that chronic stimulation of the CAP at the level of the efferent vagus nerve would attenuate hypertension in SLE. Starting at 30 and 32 weeks of age, female NZBWF1 SLE mice and NZW control mice were treated with the pharmacologic efferent vagal stimulators CNI-1493 (CNI; 8mg/kg; twice weekly; i.p.) or galantamine (GAL; 4mg/kg; daily; i.p.), or saline.
At 34 weeks of age, we measured mean arterial pressure (MAP), finding that MAP (mmHg) in SLE mice was elevated compared to controls (139.83 ± 4.56 vs. 120.70 ± 2.96; n=4-6/group, p = 0.002), while the rise in MAP was prevented by CNI (134.45 ± 3.07) and GAL (129.25 ± 3.97) in SLE mice. We further hypothesized that splenocytes isolated from SLE mice conditioned by efferent vagal stimulation would release fewer pro-inflammatory cytokines in the presence of norepinephrine, which stimulates splenic β2 adrenergic receptors. We incubated isolated splenocytes for 24 hours at 37°C with and without norepinephrine (100 μM), then measured pro-inflammatory cytokines in the supernatant via ELISA. Compared to control mice, splenocytes from SLE mice secreted 70.7% and 146.5% higher concentrations of IL-6 and TNF-α (8.24 vs. 4.83 and 2.79 vs. 1.13 pg/mL, respectively; n=2/group) in the presence of norepinephrine. Compared to saline-treated SLE mice, splenocytes from CNI and GAL-treated SLE mice released fewer cytokines when incubated with norepinephrine (8.24 vs. 5.31 and 5.79 pg/mL IL-6; 2.79 vs. 2.18 and 0.81 pg/mL TNF-α; n=2/group). These in vivo and in vitro data suggest that stimulation of the CAP at the level of the efferent vagus may promote anti-inflammatory splenocyte activity, which may be protective against hypertension in the setting of chronic inflammation.

G.S. Pham: None. A.S. Fairley: None. K.W. Mathis: None.

Funding: Yes
Funding Component: National Center 089

Dopamine D2 Receptor is Associated with Inverse Salt Sensitivity

Peng Xu, John J Gildea, Univ of Virginia, Charlottesville, VA; Pedro A Jose, George Washington Univ Sch of Med, Washington DC; Robert M Carey, Robin A Felder, Univ of Virginia, Charlottesville, VA

Our previous studies of salt sensitivity of blood pressure have demonstrated that approximately 11% of study participants have a paradoxical increase in blood pressure (> or = to 7-mm Hg) on a low NaCl diet (defined as inverse salt sensitivity (ISS)). However the mechanisms responsible for this effect are not known. We demonstrated that single nucleotide polymorphisms (SNPs) in the dopamine type 2 receptor (D2R) (RS6276 and 6267) are highly associated with ISS (P values of 1.0×10−2 and 3.8×10−2 with odds ratios of 0.32 and 0.48 in unadjusted regression models, respectively). The C allele at both sites confers protection. The D2R is strongly expressed throughout the cytoplasm of proximal tubule cells in human kidney tissue slices. We also cultured RPTC from the urine from 4 salt resistant (SR) and 3 ISS participants enrolled in our clinical salt sensitivity studies. We hypothesize that D2R containing SNPs have altered receptor expression, and altered signaling compared to wild type controls. ISS participants were homozygous variant for the two D2R alleles and showed more D2R expression than SR RPTC heterozygous variant (HV) for the two alleles (ISS: 1.166±0.059 n=3 vs SR: 0.969±0.024 n=4, P<0.05, t-test). D2R expression was increased when the ISS cells were stimulated by a non-selective D2R agonist bromocriptine to a greater extent in the D2R SNP cell lines (ISS: VEH 1.166±0.059, vs bromocriptine 1.474 ± 0.040, n=3, P<0.05, t-test). Using the ROS reagent dihydroethidium, there was found to be more ROS products in ISS cells than SR cells when stimulated under low salt (ISS: 1.145 ± 0.053, n=3 vs SR: 0.722 ± 0.101, n=4, P<0.05, t-test). We used a highly selective D2R agonist (sumanirole) to stimulate wild-type and SNPed cells, and the results demonstrated no effect in the cells with wild type D2R but an increase in...
ROS in cells heterozygous for the D2R SNPs (SNP: VEH 38,364±1,266, sumanirole 50,926 ± 3,310, VS WT: VEH 34,562±1,831 sumanirole 34,435 ± 1,614 RFU n=12, P<0.05, t-test) consistent with the higher expression of D2R found in ISS urine cells. We hypothesize that SNPs in the D2R lead to increased reactive oxygen species which has previously been associated with renal fibrosis and hypertension.

P. Xu: None. J.J. Gildea: None. P.A. Jose: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5P01HL074940-12. R.M. Carey: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5P01HL074940-12. R.A. Felder: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5P01HL074940-12.

Funding: No

Funding Component:

091

High Salt Promotes Conversion of Human Monocytes Into Dendritic Cells via Formation of Immunogenic Isoketals


High salt intake and inflammation are implicated in the genesis of hypertension. Recently it has become clear that sodium can accumulate in the interstitial space in concentrations exceeding that of the plasma, and that these high salt (HS) concentrations can be pro-inflammatory. Our laboratory recently published a new pathway in which increased oxidative stress in dendritic cells (DCs) leads to formation of isoketal-modified proteins which act as neo-antigens to activate T cells. We hypothesized that increasing sodium chloride (NaCl) in excess activates antigen presenting cells via formation of immunogenic isoketals. We exposed monocytes from human volunteers to normal physiological NaCl (NS: 150 mM/L), elevated NaCl concentrations (HS: 190 mM/L), or an equiosmolar concentration of mannitol. We found that exposure of human monocytes to high salt, but not mannitol, caused a 2-fold increase in formation of isoketal-modified proteins. This was associated with an increase in activation marker CD86 (NS: 466 ± 192 vs HS: 596 ± 324 MFI p<0.05) and production of inflammatory cytokines IL-6, IL-β and TNF-α. Interestingly, these cells expressed surface markers indicative of transformation to DCs, as evidenced by their acquisition of surface marker CD83. In additional immunofluorescence studies, we found that monocytes exposed to HS for 7 days acquire a DC like morphology. Moreover, using flow cytometry, we confirmed that high salt exposure causes these cells to lose the monocyte marker CD14 (NS: 41.1 ± 15.4 vs HS: 19.9 ± 6.4 MFI; p<0.05), and gain the DC marker CD209 (NS: 24.2 ± 1.0 vs HS: 49.3 ± 0.7 MFI; p<0.001). None to these effects were mimicked by mannitol and scavenging of isoketals during high salt exposure. High salt dramatically increased mRNA expression of GM-CSF (NS: 758 ± 440.8 vs HS: 5476 ± 2268 MFI; p<0.05), IL-4 NS: 1868 ± 560.6 vs HS: 4867 ± 1152 MFI; p<0.05) and Flt3 (NS: 2526 ± 636.8 vs HS: 10014 ± 2370 MFI; p<0.05), which are known to mediate monocyte conversion to DCs. Thus, we have defined a novel pathway whereby high NaCl concentrations lead to transformation of monocytes to DCs due to increased formation of immunogenic isoketals. These observations provide insight into how elevated sodium environments lead to an inflammatory state.
Novel Mechanism of Salt-sensitive Hypertension: CD8+ T Cells Stimulate Sodium Chloride Co-transporter NCC in Kidney

Shengyu Mu, Yunmeng Liu, Sung Rhee, Jessica Webber, Beixiang He, Tonya Rafferty, Univ of Arkansas for Medical Sciences, Little Rock, AR

Recent studies suggest a role for T lymphocytes in hypertension. However, whether T cells contribute to renal sodium retention is an important question, which if answered, could reveal a critical relationship between adaptive immunity and pathogenesis of salt-sensitive hypertension. In the present study, we propose a novel mechanism of salt-sensitive hypertension via the distal nephron: that in salt-sensitive hypertension, renal infiltrating T cells interact with distal convoluted tubule cells (mDCTs), which leads to up-regulation of the sodium-chloride-co-transporter (NCC), and thus enhancement of sodium retention. In vivo tests used DOCA-salt mice (SBP~190mmHg on a high salt diet) and T cell-adoptive transferred mice (SBP~170mmHg on a high salt diet). Results confirmed enhanced NCC & p-NCC expression (>2.5X) in the kidneys of both mouse models. We found significantly more T cells infiltrating the kidneys of DOCA-salt & T cell-transferred mice compared to their controls. It is noteworthy that in both models a great number of CD8+ T cells (CD8Ts) in the renal tubulointerstitium surround DCT segments which have higher levels of NCC expression. Direct contact between these two cell types was confirmed by super-resolution structured illumination microscopy. In vitro, we co-cultured mDCTs with T cells, and found that CD8Ts, but not CD4Ts, significantly increased NCC expression (>3X) in mDCTs via “direct cell-cell contact”. An intracellular sodium-indicator detected higher NCC-mediated sodium uptake (>1.5X) in mDCTs treated with CD8Ts compared to untreated controls. Moreover, these effects were mediated by increased (>2.2X) functional chloride channel Clc-k in the cell membrane. Clc-k knockdown by siRNA blunted chloride efflux and abolished CD8T-induced NCC activation and sodium retention in mDCTs. Taken together, our findings suggest a novel role for CD8+ T cells in enhancement of salt retention contributing to salt-sensitive hypertension: that CD8+ T cells directly contact mDCTs, stimulate NCC by upregulating the chloride channel Clc-k on the mDCT plasma membrane, thereby increasing chloride efflux, which leads to compensatory chloride influx via NCC activation at the cost of increasing sodium influx.

S. Mu: None. Y. Liu: None. S. Rhee: None. J. Webber: None. B. He: None. T. Rafferty: None.

Funding: Yes
Funding Component: South Central Affiliate (Arkansas, New Mexico, Oklahoma & Texas)
It is well known that blood pressure (BP) responses to dietary sodium intake vary among individuals (salt-sensitivity and salt-resistance). However, it is unknown whether salt-sensitivity and salt-resistance predict the risk of hypertension. We conducted a dietary sodium intervention study among 1,906 Han Chinese in 2003-05 and followed the study participants in 2008-09 and 2011-12. The dietary intervention included a 7-day low-sodium feeding (51.3 mmol/day) and a 7-day high-sodium feeding (307.8 mmol/day). Three BP measurements were obtained during each of the 3 days of baseline observation and on days 5, 6, and 7 of each intervention period. We used latent class models to identify subgroups that share a similar underlying trajectory in BP responses to sodium intervention. Over an average of 7.4 years of follow-up, we identified 514 incident hypertension cases. The mean (standard deviation) change in systolic BP during low-sodium and high-sodium interventions according to salt-sensitive and -resistant groups are shown in the following table. In addition, age, sex, and baseline BP-adjusted and multiple-adjusted odds ratios (95% CI) of incident hypertension are shown in the following table. These data indicate that high responses or non-responses to dietary sodium intervention are related to the risk of hypertension. Furthermore, this is the first prospective cohort study to indicate that individuals with either salt-sensitivity or salt-resistance are at an increased risk for hypertension and should be targeted for dietary intervention.

| Pappa2 is Important for Determining the Nephron Number |

Vikash Kumar, Chun Yang, Aron M. Geurts, Mingyu Liang, Allen W. Cowley Jr., Medical Coll of Wisconsin, Milwaukee, WI

Pappa2 is a metalloprotease which specifically cleaves IGFBP-3 and IGFBP-5 and in turn releases IGF-1. Recently, we have shown that a subcongenic Dahl salt-sensitive (SS) rat strain containing a 0.71 Mbp of chromosome 13 which includes Pappa2 gene from salt-insensitive Brown Norway (26-P strain) is protected significantly (24 mmHg) from salt-induced hypertension (Cowley et al., 2016). Although it is recognized that Pappa2 modulates development of bone size, cranial cartilage and angiogenesis, its role in kidney development and function is unknown. The present study determined the contribution of Pappa2 to nephron development by comparing SS and 26-P rat strains. It was found that Pappa2 mRNA expression was 5-fold higher in embryonic kidney (day 20.5) of the salt-resistant 26-P rats compared with age-matched SS strain. Pappa2 mRNA expression significantly increased with age of kidney reaching a maximum at postnatal day 5 in both strains. Pappa2 mRNA expression at postnatal day 15 was found to be 9-fold higher in the kidney of 26-P compared with SS strain. Immunohistochemistry studies revealed that Pappa2 co-localized with IGFBP-5 in the ureteric bud indicating that Pappa2 could be important for ureteric branching and nephron development.
endowment. Glomerulus/mm² was therefore determined by counting total number of glomeruli in kidney sections from pups starting from P0 to P20. The salt-resistant 26-P congenic strain exhibited significantly greater nephron density 9.03 and 7.07 glo/mm² compared to 6.89 and 4.85 glo/mm² in SS rat at day P15 and P20, respectively. It appears that the Brown Norway pappa2 allele variant prevents the reduced nephron numbers observed in SS rats and thereby protects these congenic rats from salt-induced hypertension.

V. Kumar: None. C. Yang: None. A.M. Geurts: None. M. Liang: None. A.W. Cowley: None.

Funding: No

Funding Component:

095

**Ovariectomy Restores Purinergic Receptor Activation of Endothelin-dependent Natriuresis**

Eman Y Gohar, David M Pollock, Univ of Alabama at Birmingham, Birmingham, AL

We have recently reported interplay between renal medullary endothelin-1 (ET-1) and purinergic (P2) systems, which play central roles in controlling Na⁺ homeostasis in male rats. Evidence suggests that sex hormones regulate ET-1 and P2 systems. To test whether activation of the renal medullary P2 receptors promotes ET-dependent natriuresis in females and whether ovariectomy (OVX) modulates this potential interaction, we studied the effect of medullary NaCl loading on Na⁺ excretion in adult intact female and OVX SD rats in the presence and absence of P2 or ET receptor antagonism. Isosmotic saline (284 mOsmol/kg H₂O) was infused into the renal medullary interstitium during a baseline urine collection period, followed by isosmotic or hyperosmotic saline (1800 mOsmol/kg H₂O) infusion. Blood pressure, renal blood flow, urine Na⁺, K⁺ and osmolality were measured. Medullary NaCl loading significantly enhanced Na⁺ excretion in intact females and OVX (from 0.8±0.2 to 6.2±1.6 and from 0.7±0.1 to 5.6±0.8 μmol/min, respectively, n=6-8, p<0.05). The natriuretic effect of NaCl loading in intact females was not attenuated by P2 or ET receptor blockade. Whereas, intramedullary infusion of the P2 receptor antagonist, suramin, inhibited the natriuresis induced by medullary NaCl loading in OVX (from 0.4±0.2 to 0.9±0.4 μmol/min, n=6). Additionally, combined ETA/B receptor blockade (ABT-627 + A-192621) abolished the natriuretic response to medullary NaCl load in OVX rats (from 0.2±0.1 to 1.4±0.7 μmol/min, n=4). Activation of medullary purinergic (P2Y2/4) receptors by UTP infusion had no significant effect in intact females, but enhanced Na⁺ excretion in OVX rats (from 0.5±0.1 to 2.3±0.8 μmol/min, n=5, p<0.05). Combined ET A/B receptor blockade significantly inhibited the natriuretic response to UTP observed in OVX rats. These data suggest that increased medullary NaCl loading induces ET-independent and P2-independent natriuresis in intact females. In OVX, activation of medullary P2 receptors promotes ET-dependent natriuresis, similar to our previous findings in male rats and suggests that OVX restores the interplay between the renal ET-1 and purinergic (P2) signaling systems to facilitate Na⁺ excretion. Funded by AHA 15POST25090329 to EYG and P01 HL95499 to DMP

E.Y. Gohar: None. D.M. Pollock: None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)
Leptin and High Salt Diet Induce Greater Increases in Blood Pressure in Female than Male Mice

Jessica L Faulkner, Eric J Belin de Chantemele, Augusta Univ, Augusta, GA

Recent studies by our group demonstrated that leptin is a direct regulator of aldosterone secretion and increases blood pressure via sex-specific mechanisms involving leptin-mediated activation of the aldosterone-mineralocorticoid receptor signaling pathway in females and sympatho-activation in males. Although it is well accepted that females secrete more leptin and aldosterone than males, it is unknown whether leptin infusion raises blood pressure similarly in male and female mice and whether higher aldosterone levels sensitize females to salt-induced hypertension. We hypothesized that female mice would be more sensitive to leptin than males and also have a potentiated blood pressure rise in response to high salt diet compared to males. Male and female Balb/C mice were implanted with radiotelemeters for continuous measurement of mean arterial pressure (MAP) at 10 weeks of age. MAP was measured for seven days prior to feeding with a high-salt diet (HS, 4%NaCl) for seven days. Following a recovery period, animals were then implanted with osmotic minipumps containing leptin (0.9mg/kg/day) recorded for seven days. Baseline MAP was similar between males and females (101.3±2.9 vs 99.3±3.7 mmHg, n=4 and 5, respectively), however, HS diet resulted in a greater MAP increase in females (15.0±2.6 mmHg) compared to males (3.1±4.5 mmHg, P<0.05). MAP with leptin treatment was increased with leptin in females moreso than in males, however, this did not reach significance (6.8±5.8 vs 1.8±5.9 mmHg, respectively). This potential sex difference in blood pressure responses to leptin was not associated with changes in body weight (0.07±0.44 vs -0.22±0.2 g, respectively) nor changes in blood glucose (-19.67±15.06 vs -15.4±11.4 mg/dl, respectively) in males and females in response to leptin. In summary, female mice are more sensitive to HS diet-induced blood pressure increases than males. Females may be more sensitive to leptin-mediated blood pressure increases than males. Further investigation is needed to determine whether these sex differences in blood pressure responses to HS diet and leptin are mediated by aldosterone or other mechanisms.

J.L. Faulkner: None. E.J. Belin de Chantemele: None.

Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

097

Effects of Lysine-specific Demethylase 1 Deficiency on Aldosterone Production and Salt-sensitive Hypertension in Female Mice

Yuefei Huang, Tham M Yao, Paul Loutraris, Isis K Rangel, Pei Yee Ting, Jia Wei Tan, Amanda E Garza, Jose R Romero, Luminita H Pojoga, Gail K Adler, Gordon H Williams, Brigham and Women's Hosp & Harvard Medical Sch, Boston, MA

Lysine-Specific Demethylase1 (LSD1) is an epigenetic factor modulated by salt intake. Previously, we documented the male heterozygote LSD1 knockout mice (LSD1+/-) had dysregulation of aldosterone (ALDO) production on a liberal salt diet (1.6% Na+) associated with salt-sensitive hypertension. This study assessed if: 1) female LSD1+/-- mice have a similar phenotype; and 2) the effect of aging on this phenotype. Methods: Female LSD1+/-- and wild type mice (LSD1+/+) were randomly assigned for sacrifice at the ages of 18-week, 52-week, and 75-week and the following were assessed
at each time point: blood pressure (BP); plasma renin activity (PRA) and ALDO; urine albumin; and *ex vivo* ALDO production from isolated adrenal zona glomerulosa cells. **Results:** BP and urine albumin in the LSD1+/− compared to the LSD1+/+ were not different at any age (Table). However, the LSD1+/− had greater ALDO/PRA ratios at 18 weeks compared with the LSD1+/+, but lower ALDO levels and *ex vivo* ALDO production at 52 and 75 weeks. Associated with this phenotype, the LSD1+/− showed significantly higher rate of all-cause mortality than the LSD1+/+. **Conclusion:** Lack of LSD1 caused dysregulation of ALDO production in both male and female mice. But the cardiovascular outcomes are different. The LSD1+/− females in contrast to males do not develop hypertension or albuminuria even at 75 weeks of age. However, the females do die at a faster rate than the males of a variety of causes. Thus, there is considerable sexual dimorphism in the pathogenesis of cardiovascular outcomes associated with dysregulation of adrenal ALDO production mediated by lack of LSD1.

**Viknesh Selvarajah,** Kaisa Maki-Petaja, Experimental Med & Therapeutics, Univ of Cambridge, Cambridge, United Kingdom; Liliana Pedro, Sylvaine F Bruggaber, MRC Human Nutrition Res Unit, Cambridge, United Kingdom; Morris J Brown, William Harvey Res Inst, Queen Mary, Univ of London, London, United Kingdom; Carmel M McEniery, Ian B Wilkinson, Experimental Med & Therapeutics, Univ of Cambridge, Cambridge, United Kingdom

**Introduction:** Dietary sodium is an important trigger for hypertension. Animal studies show that the skin buffers dietary salt and salt-loading induces lymphangiogenesis mediated by VEGF-C, maintaining normal BP. Our primary objective was to determine whether increased skin Na+ on salt loading attenuates the expected rise in blood pressure.

**Methods:** We assessed skin and urine electrolytes, systemic haemodynamics, ambulatory BP and plasma VEGF-C in 48 healthy participants. Participants were placed on a low salt diet (70mmol sodium/day) and received placebo and slow sodium (200mmol daily for 7 days) in a randomised order. Skin Na+ and K+ concentrations were measured in mg/g tissue by inductively coupled plasma optical emission spectroscopy. Results were expressed as the ratio of Na+:K+ to correct for variability in sample hydration. Plasma VEGF-C was analysed by ELISA.

**Results:** Mean age was 30 ± 8 years (24 male). 24-hr urine Na+ was higher following salt than placebo in males (221.9 ± 16.5 vs. 86.3 ± 10.3mmol, p < 0.001) and females (227.1 ± 19.9 vs. 60.0 ± 6.8mmol, p < 0.001). In males, skin Na+:K+ was higher after salt loading (2.87 ± 0.12 vs. 2.59 ± 0.09 mg/g, p = 0.008), but not significantly different in females (3.36 ± 0.12 vs. 3.23 ± 0.10 mg/g, p = 0.49). Females showed a significant increase in 24-hr MAP with salt loading (88 ± 1 vs. 85 ± 1 mmHg, p=0.004) but not males (90 ± 2 vs. 90 ± 2 mm Hg, p = 0.91). In
males only, skin Na⁺:K⁺ correlated with supine MAP post-placebo (r = 0.52, p = 0.004) and post-salt (r = 0.51, p = 0.01). No significant change was noted in plasma VEGF-C.

**Conclusions:** Salt loading causes an increase in BP in healthy females but not in males, whilst skin sodium rises in males. This indicates that the skin may buffer the haemodynamic consequences of increased salt intake in a gender-specific manner.

**V. Selvarajah:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; British Heart Foundation, National Institute for Health Research. **K. Maki-Petaja:** None. **L. Pedro:** None. **S.F.A. Bruggraber:** None. **M.J. Brown:** None. **C.M. McEniery:** None. **I.B. Wilkinson:** None.

**Funding:** No

**Funding Component:**

099

**Functional Remodeling of the Renal Vasculature Precedes the Establishment of Salt-sensitive Hypertension in Eln-deficient Mice**

Elizabeth A Owens, Li Jie, Beverly Reyes, Elisabeth J Van Bockstaele, **Patrick Osei-Owusu,** Drexel Univ Coll of Med, Philadelphia, PA

Vascular stiffening due to elastin deficiency is a leading risk for hypertension and chronic kidney disease (CKD). However, the mechanisms by which elastin deficiency is involved in the pathogenesis of hypertension and/or CKD are poorly understood. Here, we used elastin heterozygous mice (Eln+/−), an animal model of elastin insufficiency, to test the hypothesis that renal dysfunction due to elastin deficiency occurs independently of and precedes the development of hypertension in this mouse model.

We assessed blood pressure (BP) and renal hemodynamics in 30-day (P30) and 12-week old anesthetized male and female mice. At P30, mean blood pressure of Eln+/− was similar to wild type (WT) controls (Eln+/−, 79 ± 5 vs. WT, 69±3 mmHg, P = 0.06); however, renal blood flow was lower (Eln+/− 2.9 ± 0.2 vs. WT 4.0 ± 0.5 mL/min/g KW, P = 0.03) whereas renal vascular resistance (RVR; Eln+/− 29 ± 3 vs. WT 18 ± 3 mmHg/mL/min/g KW, P = 0.03) was augmented at baseline in Eln+/− mice. At 12 wks old, RVR remained elevated while filtration fraction was higher in male Eln+/− relative to WT mice (Eln+/− 44 ± 3 vs. WT 38±5 % P = 0.07). Eln+/− mice showed isolated systolic hypertension that was evident only at nighttime (Eln+/− 136 ± 2 vs. WT 112 ± 6 mmHg, P <0.01). Acute salt loading with 6% dietary sodium increased daytime systolic blood pressure only in male Eln+/− mice (Eln+/− 118 ± 5 vs. WT 102 ± 6 mmHg, P = 0.03), causing a rightward shift and blunted slope of the pressure-natriuresis curve. Renal interlobar artery basal tone and myogenic response to increasing intraluminal pressure at P10 were similar (Eln+/− 78 ± 3 vs. WT 67 ± 6 % P = 0.06) whereas they were augmented at P30 (Eln+/− 63 ± 4 vs. WT 49 ± 6 % P = 0.05) and at 12 wks old in Eln+/− mice (Eln+/− 50 ± 2 vs. WT 33 ± 3 % P < 0.01), and normalized by the AT1R blocker, candesartan (Eln+/− 22 ± 9 vs. WT 8 ± 5 % P = 0.10).

We conclude that AT1R mediates augmented mechanotransduction and renal vascular dysfunction due to Eln insufficiency that in turn contribute to altered renal sodium handling and increased BP. Such prolonged systemic BP elevation leads to glomerular structural damage due to high renal perfusion pressure. Therefore, therapies that target the AT1R to control BP in patients with elastin deficiency may be beneficial in preventing hypertension-evoked kidney damage.
Observed studies suggest that thiazide-type diuretics reduce fracture risk compared to other antihypertensive medications. The effects of calcium channel blockers (CCB) and angiotensin converting-enzyme inhibitors (ACEi) on fracture risk have not been well studied. We examined the relationship of antihypertensive drug therapy and hip and pelvic fracture hospitalizations in the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). It included >33,000 participants randomized to the thiazide-type diuretic chlorthalidone (C), ACEi lisinopril (L), or CCB amlodipine (A) as first line hypertension (HTN) therapy. Mean follow up was 4.9 years during the randomized phase (in-trial), and 5 additional years after the conclusion of the trial (post-trial) using linkage to national data bases. Risks of hip and pelvic fractures for L and A relative to C were derived from Cox models. There were 341 hip and pelvic fractures in-trial. Participants assigned C had the lowest risk and those assigned L the highest (Figure 1a). The adjusted risk for L compared to C was 1.33 (95% CI 1.02-1.73; p=.04). Participants assigned A had intermediate risk compared to C (HR 1.22, 95% CI 0.93-1.59). During the combined in-trial and post-trial periods (Figure 1b), there were 646 fractures; the results were similar to the in-trial results, although differences were not statistically significant. Participants randomized to C continued to have the lowest risk of fractures after in-trial period, suggesting a legacy effect from prior C use. These findings have public health importance given the high prevalence of HTN in older adults and the widespread use of A and L in older adults.
(Calhoun PI). **R. Puttnam**: None. **B. Davis**: None. **S. Pressel**: None. **P. Whelton**: None. **W. Cushman**: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; I am co-investigator for a glycemia study (but also measuring ABPM) with Boehringer-Ingelheim, on Steering Committee for an international diabetes outcome trial (REWIND) sponsored by Eli Lilly, and p. **G. Louis**: None. **K. Margolis**: None. **J. Williamson**: None. **A. Ghosh**: None. **P. Einhorn**: None. **J. Barzilay**: None.

Funding: No

Funding Component: 101

**Alms1 (Alstrom Syndrome 1), a Novel Gene Involved in Blood Pressure Regulation, Renal Na Handling and Thick Ascending Limb (TAL) Function**

Ankita Bachhawat Jaykumar, Wayne State Univ/ Henry Ford Hosp, Detroit, MI; Paulo Caceres, Gustavo Ares, Henry Ford Hosp, Detroit, MI; William H Beierwaltes, Wayne State Univ/Henry Ford Hosp, Detroit, MI; Pablo A Ortiz, Wayne State Univ/ Henry Ford Hosp, Detroit, MI

Single nucleotide polymorphisms in the Alstrom syndrome 1 (ALMS1) gene are associated to hypertension, renal dysfunction, and obesity in the general population. The role of ALMS1 in regulating blood pressure or renal Na handling is unknown. In a proteomics screen, we identified ALMS1 as an interacting protein with the Na/K/2Cl cotransporter NKCC2. NKCC2 mediates NaCl reabsorption by the TAL. Thus, we hypothesized that ALMS1 regulates NKCC2 endocytosis and activity as well as blood pressure. First, we confirmed the expression of ALMS1 in isolated perfused TALs. To study ALMS1 function, we generated ALMS1 knockout (KO) rats in collaboration with the rat genome editing consortium at MCW and confirmed deletion of the ALMS1 gene. We found that 3 month old ALMS1 KO rats are hypertensive compared to wild type littermates (ALMS1 MAP: 141 ± 5 mmHg vs WT: 99 ± 6 mmHg, p<0.005) fed a normal Na diet. We measured surface and intracellular NKCC2 and found a higher percentage of NKCC2 at the surface in TALs from ALMS1 KO (ALMS1: 13.8 ± 1.2% vs WT: 9.1 ± 1.0%, p<0.05, n=6). The increase in surface NKCC2 is due to lower endocytosis because the rate of NKCC2 internalization was lower in TALs from ALMS1 KO (ALMS1: 28.2 ± 2.8% over 20 min, p<0.01, n=6). To study NKCC2-mediated Na transport in vivo, we measured bumetanide-induced natriuresis and diuresis. ALMS1 KO rats exhibited higher bumetanide-induced natriuresis (ALMS1: 1292 ± 65 vs WT: 564 ± 31 µmoles, p<0.01, n=5) and diuresis (ALMS1: 3.1 ± 0.32 vs WT: 1.6 ± 0.13 ml, p<0.05), indicative of higher TAL Na reabsorption. To study if this decreases the ability to excrete a volume and Na load in ALMS1 KO, we instrumented anesthetized rats and measured sodium excretion (UNaV) and urine volume (UV) over 150 min after an acute saline load. We found that the cumulative UNaV was lower in ALMS1 KO rats (ALMS1: 72 ± 38 vs WT: 219 ± 55 µmoles, p<0.05, n=8) as was the UV (ALMS1: 0.70 ± 0.24 vs WT: 1.78 ± 0.39 ml, p<0.05). We conclude that deletion of ALMS1 decreases NKCC2 endocytosis and increases TAL Na reabsorption. Thus, the hypertension observed in ALMS1 KO rats may be in part due to higher renal Na reabsorption. It is not known whether the expression of ALMS1 protein is decreased in hypertensive patients or lowered by dietary factors that increase BP in humans.


Funding: Yes
Role of Renal Tubular NHE3 for Blood Pressure and Salt Homeostasis

Timo Rieg, UCSD & VASDHS, San Diego, CA; Soren B Poulsen, Aarhus Univ & VASDHS, San Diego, CA; Jessica A Dominguez Rieg, Bastyr Univ & VASDHS, San Diego, CA; Robert A Fenton, Aarhus Univ, Aarhus, Denmark

The Na+/H+ exchanger isoform 3 (NHE3) is expressed in the intestine and the kidney where it facilitates Na+ absorption and H+ secretion. The importance of NHE3 in the kidney for NaCl homeostasis and blood pressure regulation, relative to the intestine, is not known. To investigate this, we examined kidney-specific NHE3 knockout mice (NHE3loxloxPax8Cre) and control mice (Con, NHE3loxlox) under normal, low or high dietary NaCl intake. Under normal dietary NaCl intake, no significant differences were detected between genotypes in body weight, fluid or food intake, blood pH and plasma Na+, K+ or aldosterone levels. However, urinary pH was significantly elevated in NHE3loxloxPax8Cre mice and GFR was significantly lower (457±20 vs 358±17 µl/min, P<0.05). High NaCl intake (4% for 10 days) had no impact on plasma Na+ in Con mice; but plasma Na+ concentrations in NHE3loxloxPax8Cre mice were susceptible to the effects of low NaCl (<0.01% for 10 days) (-3.9±1.0 mM, P<0.05) or high NaCl intake (+2.2±0.6 mM, P<0.05) compared to baseline conditions. Low NaCl diet decreased plasma K+ levels in Con mice (-0.5±0.2 mM, P<0.05) but to a significantly greater amount in NHE3loxloxPax8Cre mice (-1.2±0.1 mM, P<0.05). Plasma aldosterone was not significantly different between Con and NHE3loxloxPax8Cre mice under low (345±96 vs 498±78 pg/ml) or high NaCl intake (60±19 vs 86±20 pg/ml). Low NaCl intake decreased GFR in Con (-110±13 µl/min, P<0.05) and NHE3loxloxPax8Cre (-99±8 µl/min, P<0.05) mice, whereas high NaCl intake was without effect on GFR in either genotype. Dietary NaCl did not affect blood pressure in Con mice (low NaCl: 102±3; high NaCl: 102±3 mmHg); however, blood pressure was significantly lower in NHE3loxloxPax8Cre mice and salt-sensitive (low NaCl: 81±2; high NaCl: 91±2 mmHg, P<0.05). Alterations in the abundances of several Na+ transport proteins within the kidney tubule system were also apparent in NHE3loxloxCre mice under different dietary conditions. In conclusion, renal NHE3 is required to maintain blood pressure and steady state plasma Na+ levels when dietary NaCl intake is modified.

T. Rieg: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIDDK. S.B. Poulsen: None. J.A. Dominguez Rieg: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Bastyr University Seed Grant. R.A. Fenton: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Danish Medical Research Council; Lundbeck, Novo Nordisc and Carlsberg Foundation.

Funding: Yes
Funding Component: Western States Affiliate (California, Nevada & Utah)
The extracellular domain of (pro)renin receptor (PRR) is cleaved to produce a 28 kDa soluble receptor (sPRR) which is detected in biological fluid and elevated under certain pathological conditions. Our recent work suggests that sPRR derived from collecting duct intercalated cells acts in a paracrine fashion to regulate water transport in the principal cells. The present study attempted to further define the role of sPRR in vasopressin (AVP) signaling with emphasis on V2R regulation. In primary rat IMCD cells, treatment with a recombinant sPRR termed as sPRR-His at 10 nM for 12 h induced a 2.8-fold increase in V2R protein and a 2-fold increase in V2R mRNA. Following AVP treatment, V2R protein expression was increased by 3-fold, which was blunted by a PRR antagonist (PRO20) and a PRR neutralizing antibody. Mice with CD-specific (CD PRR KO) developed a medium level of diabetes insipidus (urine volume: KO: 2.2±0.4 versus Floxed: 1.2±0.3 ml/day; \( p<0.05 \)), accompanied with a 60% reduction of renal V2R protein and a 25% reduction of urinary sPRR excretion. Administration of sPRR-His for 3 d almost completely rescued the polyuria phenotype of CD PRR KO mice (urine volume: KO+sPRR-His: 1.6±0.3 ml/day; \( p<0.05 \)) and restored renal expression of V2R and AQP2, as well as AVP sensitivity. In contrast, the downregulation of NKCC2 expression in the null mice was unaffected by sPRR-His infusion nor was the upregulation of autophagosome marker microtubule-associated protein 1A/1B-light chain 3 (LC3b). Together, our data suggests that sPRR selectively targets the CD to determine V2R expression and hence AVP sensitivity and urine concentrating capability, independently of autophagosome accumulation.


Funding: No

Funding Component: 104

Dynamic Autoregulation of Glomerular Capillary Pressure

Scott C Thomson, Univ of California and VAMC, San Diego, CA

It is generally accepted that renal blood flow (RBF) autoregulation is mediated by myogenic and tubuloglomerular feedback responses acting on the pre-glomerular resistance. If this is so, then autoregulation of RBF and glomerular capillary pressure (PGC) should change in the same direction throughout an autoregulatory step response. We computed autoregulatory step responses from time series recordings of arterial blood pressure (BP) and RBF (Transonics) blood flow or tubular stop-flow pressure (micropuncture), which is a surrogate for PGC in Wistar-Froemter rats fed for one week on low or high salt diets (n=6-10). Autoregulatory step responses were generated from time series by an algorithm that treats BP
as a leading indicator of RBF or PGC and uses the projection theorem to solve for the impulse response which is integrated to obtain the step response. Step responses shown in the figure represent the uncompensated changes in RBF and PGC (mean ± SEM) following a 1 mmHg BP step. The data clearly reveal that the time courses of RBF and PGC differ such that changes in RBF cannot predict changes in PGC. This implies that the renal hemodynamic response to a blood pressure disturbance is not confined to the pre-glomerular resistance. Furthermore, the participation of post-glomerular resistance in the autoregulatory response is sensitive to dietary salt such that PGC is more sensitive to BP on low salt diet.

Erwin T Cabacungan, Pediatrics, Medical Coll of Wisconsin, Milwaukee, WI; Aron M Geurts, David L Mattson, Liang Mingyu, Ctr of Systems Molecular Med, Physiology, Medical Coll of Wisconsin, Milwaukee, WI

We have shown that maternal exposure to Casein-based AIN-76A diet [SS/JrHsdMcwi (SS/Mcw) rats] compared to grain-based 5L2F diet [SS/JrHsdMcwiCrl (SS/Crl) rats] during the gestational-lactational period exacerbates the development of salt-induced hypertension and renal injury in adult SS rats. We hypothesized that SS/Mcw rats will have significantly fewer glomeruli than SS/Crl rats, and determined its potential mechanisms. At different time points during development [embryonic day 20 (E 20), day of life (DOL) 14, 21, 56]: glomerular counts were determined by maceration method and by glomerular density (total glomerular number/renal cortex area); apoptosis was measured by TUNEL assay; RT-PCR, Western Blot and immunostaining were used to examine the expression level of genes involved in the apoptotic pathway and unfolded protein response (UPR). We found lower glomerular counts in SS/Mcw compared to SS/Crl rats at DOL 21 [26048±1423 per kidney vs. 34766±1821 (p<0.001)] and DOL 56 [15717±2052 vs. 27999±3362 (p <0.001)], and lower glomerular densities [DOL 21, 10.8±1.0/mm2 vs. 13.2±1.6 (p<0.001); DOL 56, 2.4±0.1 vs. 3.6±0.2 (p < 0.001)]. Glomerular density was not different between SS/Mcw and SS/Crl at DOL 14 when nephrogenesis is complete. We found higher glomerular apoptosis in SS/Mcw compared to SS/Crl rats at DOL 14 [9.5±1.4 per 100 glomeruli vs. 5.1±1.8 (p<0.01)] and DOL 21 [5.2±0.7 vs. 3.6±0.5 (p <0.01)]. mRNA levels of several genes involved in intrinsic apoptotic pathway and UPR were significantly upregulated in SS/Mcw rats compared to SS/Crl rats at different time points. Significantly lower UPR-related protein

S.C. Thomson: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Research grants from Merck and Pfizer on subjects unrelated to this abstract.

Funding: No

Funding Component: 105

Maternal Diet Leads to Nephron Count Differences After Postnatal Day 14 and Time-dependent Differences in Apoptosis and Unfolded Protein Response in Dahl Salt-sensitive Rats
expression in SS/Mcw compared to SS/Crl rats was detected for JNK and GRP78 (DOL 14 and 56) and CHOP (DOL 56). Significantly higher UPR-related protein expression in SS/Mcw compared to SS/Crl rats were detected for GRP78 (E 20), JNK (DOL 21) and Caspase 12 (DOL 14, 21 and 56). Caspase 12 was located in the glomeruli in DOL 21 and 56 kidneys. Our results show marked decrease of glomerular counts with age in SS/Mcw compared to SS/Crl after DOL 14. The time-dependent differences in the expression of apoptosis and UPR-related genes leading to glomerular apoptosis may be a potential mechanism for the nephron count differences between SS/Mcw and SS/Crl.


Funding: No
Funding Component: 106

Signaling Mechanisms Affecting Nitric Oxide Production in Glomerular Podocytes

Oleg Palygin, Daria V. Ilatovskaya, Vladislav Levchenko, Bradley T. Endres, Aron M. Geurts, Alexander Staruschenko, Medical Coll of Wisconsin, Milwaukee, WI

While Nitric Oxide (NO), a potent vasodilator and vital signaling molecule, has been shown to contribute to the regulation of glomerular ultrafiltration, its role in podocytes during the pathogenesis of salt-sensitive hypertension has not yet been thoroughly examined. Recent studies have demonstrated that the deficiency of eNOS (the enzyme responsible for synthesizing NO) exacerbates renal injury and accelerates development of proteinuria and glomerulosclerosis. Considering this, we hypothesized that the podocytes of hypertensive animals would exhibit reduced NO production in response to various paracrine factors and this directly contributes to proteinuria. To test this, we isolated glomeruli from the kidneys of Dahl salt-sensitive (SS) rats fed either a high salt (HS; 4% NaCl, 3 weeks) or low salt (LS; 0.4% NaCl) diet and loaded podocytes with a combination of NO and Ca²⁺ ionophores (DAF-FM and Fura Red, respectively). Changes in fluorescence were observed with the use of confocal microscopy in response to adenosine triphosphate (ATP), angiotensin II (Ang II), and H₂O₂. Application of Ang II or H₂O₂ resulted in activation of both NO and [Ca²⁺]; fluorescent transients which were significantly elevated in the soma and foot processes of podocytes of LS fed rats. In contrast, ATP specifically activated only changes in [Ca²⁺], but did not have any effects on NO production. Ang II treatment also caused hypertrophy of the podocytes, whereas ATP had no effect on cell volume (41.1±7.7 vs. 0.1±3.6% increase for Ang II and ATP, respectively; P<0.05). Collectively, our results show that in contrast to [Ca²⁺], which is modulated by all studied paracrine molecules, NO is produced by podocytes only in response to Ang II and H₂O₂, but not ATP. SS rats fed a HS diet for 3 weeks demonstrated impaired NO production; the response to Ang II or H₂O₂ on HS contained only 23.7±6.6 and 43.4±28.4% of total effects on LS, respectively (P<0.05). Therefore, when fed a HS diet, SS podocytes had an impaired NO response to Ang II or oxidative stress, suggesting that NO signaling is dysfunctional and likely contributes to the development of kidney injury.

O. Palygin: None. D.V. Ilatovskaya: None. V. Levchenko: None. B.T. Endres: None. A.M. Geurts: None. A. Staruschenko: D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Eli Lilly.

Funding: Yes
Funding Component: National Center
107
**Mir-431 as a Potential Master Regulator in Angiotensin II-induced Vascular Injury**

**Kugeng Huo**, Tlili Barhoumi, Julio C. Fraulob-Aquino, Lady Davis Inst of the Jewish General Hosp, Montreal, QC, Canada; Chantal Richer, Mathieu Lajoie, Daniel Sinnett, Div of Hematology-Oncology, Res Ctr, CHU Ste-Justine, Montreal, QC, Canada; Pierre Paradis, Ernesto L. Schiffrin, Lady Davis Inst of the Jewish General Hosp, Montreal, QC, Canada

**Objective:** Vascular injury is an early manifestation and a cause of end-organ damage in hypertension. microRNAs (miRNAs) play an important role in cardiovascular disease, but their implication in vascular injury remains unclear. We aim to use RNA sequencing (seq) and a systems biology approach to identify master regulators that mediate global gene expression changes in the course of vascular injury.

**Methods and Results:** Ten week-old male C57BL/6 mice were infused or not with angiotensin (Ang) II (1 µg/kg/min, SC) for 14 days. Blood pressure (BP) was measured by telemetry. Total RNA was extracted from the mesenteric vasculature for total RNA and small RNA-seq. Differentially expressed (DE) miRNAs (23 up and 12 down) and mRNAs (550 up and 256 down) were identified (1.5-fold, \(q<0.05\)). Molecular networks were constructed to integrate predicted interactions between DE miRNAs and inversely expressed DE mRNAs and between DE transcription factors (TF) and DE genes. Gene enrichment analysis revealed DE mRNAs involved in extracellular matrix (ECM) and developmental processes regulated by DE miRNAs (\(q<1.5E-11\)). Seventeen upregulated miRNAs are located in the miRNA cluster of the Dlk1-Dio3 region that is highly conserved in humans, 9 of which had expression levels correlated with BP (\(P<0.05\)). Among those 9, miR-431 that ranked first as DE miRNA (\(q<0.0005\)) and is 100% conserved in humans, and is a conserved putative DE target, a BP-correlated (\(P<0.05\)) TF ETS homologous factor (Etf), which regulates numerous ECM genes including collagen type I α1 (Col1a1), were selected for functional studies. Transfection of a miR-431 mimic in human aortic smooth muscle cells (HASMCs) decreased Etf (0.1±0.1-fold, \(P<0.001\)) and increased Etf-suppressing target Col1a1 (1.7±0.5-fold, \(P<0.001\)) mRNA levels. Transfection of a miR-431 inhibitor caused reciprocal effects (\(P<0.05\)). Etf siRNA knockdown increased Col1a1 (1.2±0.1-fold, \(P<0.001\)) mRNA levels.

**Conclusions:** Ang II infusion altered expression of miRNAs in the Dlk1-Dio3 cluster and genes involved in ECM and developmental processes. miR-431 targets TF Etf, which leads to increased Col1a1 in HASMCs. miR-431 may act as a master regulator for vascular injury and could be a potential therapeutic target.


Funding: No

Funding Component: 108

**Relative Contributions of Arterial Stiffness and Hypertension to CVD Risk: The Framingham Heart Study**


**Introduction:** The presence and implications of abnormal arterial stiffness, a potential
independent predictor of outcomes, in community-dwelling treated hypertensives is unknown. Furthermore, limited data exist regarding the risk of cardiovascular disease (CVD) associated with arterial stiffness across the entire range of blood pressure. **Methods:** We measured carotid-femoral pulse wave velocity (PWV) and classical CVD risk factors in participants of The Framingham Offspring Cohort. The participants were divided into 4 groups according to hypertension (yes/no), and PWV status (high/low based on age- and sex-specific median values) and followed up for CVD events (CVD death, myocardial infarction, unstable angina, heart failure, and stroke). **Results:** We studied 2127 community-dwelling individuals (60 years, 57% women). 60% (233 of 390) of controlled and 90% (232 of 258) of uncontrolled treated hypertensives had high PWV. The multivariable-adjusted risk for CVD events (n=248, median follow-up 12.6 years; Figure) rose from normotension with low PWV (reference) to normotension with high PWV (hazard ratio [HR] 1.33, 95% confidence interval [95% CI] 0.86-2.05) and from hypertension with low PWV to hypertension with high PWV (HR 1.53, 95% CI 1.00-2.34) to hypertension with high PWV (HR 2.31, 95% CI 1.58-3.36). **Conclusions:** A substantial proportion of treated hypertensives have high arterial stiffness, a finding that may explain some of the notable residual CVD risk associated in this group. High PWV is associated with a trend towards increasing CVD risk in both non-hypertensives and hypertensives supporting the use of arterial stiffness measurements in both populations.
Arterial stiffness and enhanced wave reflections independently predict cardiovascular risk. Wave reflections augment central (aortic) pulse pressure (PP), an index of arterial stiffness, and systolic pressure. Increased wave reflections and PP have previously been associated with endothelial dysfunction in hypertensive and healthy middle-aged adults. The study objective was to determine whether endothelial dysfunction is associated with PP and other measures of vascular stiffness in young normotensive and prehypertensive subjects. We measured office, central, and 24-hour measurements in 102 (64 female, 38 male) non-hypertensive, non-diabetic participants. Endothelial function was assessed non-invasively using post-ischemic reactive hyperemia with strain-gauge plethysmography. The racially diverse subject pool was comprised of 60% Caucasians, 18% African Americans, and 24% Asians, with mean age 30, mean BMI 25.6, mean office SBP/DBP = 110 ± 13 mm Hg/70 ± 9 mm Hg. Endothelial function was highly associated with office (β= - 4.2 mm Hg, p<0.001) and 24-hour PP (β= - 1.4 mm Hg, p=0.008), along with central measures of wave reflection: ((augmentation pressure (β= - 2.1 mm Hg), augmentation index (β= - 3.7): both p<0.001). Beta values correspond to the change noted for one standard deviation in endothelial function. In conclusion, endothelial dysfunction is significantly and consistently associated with arterial stiffness and increased wave reflections in young non-hypertensive adults. Identification of endothelial dysfunction in otherwise healthy young individuals may provide an opportunity to reduce vascular stiffness and associated cardiovascular risk.

C. Cheng: None. R. Townsend: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Fukuda. E. Honoraria; Modest; UpToDate. G. Consultant/Advisory Board; Modest; Medtronic.

J.A. Chirinos: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; American College of Radiology Network, Fukuda Denshi, Bristol-Myers Squibb, Microsoft Research and CVRx Inc., C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Atcor Medical. G. Consultant/Advisory Board; Modest; Bristol-Myers Squibb, OPKO Healthcare, Fukuda Denshi, Microsoft Research, Merck, AstraZeneca/Fibrogen and Vital Labs. S. Keith: None.

Funding: No

Funding Component: 110

Vascular Smooth Muscle Sirtuin-1 Protects Against Diet-Induced Aortic Stiffness

Jessica Fry, Leona Al Sayah, Robert Weisbrod, Isabelle Van Roy, Xiang Weng, Richard Cohen, Markus Bachschmid, Francesca Seta, Boston Univ Sch of Med, Boston, MA
Arterial stiffness (AS), a major cardiovascular risk factor, develops within two months in mice fed a high fat, high sucrose diet (HFHS), serving as a model of human metabolic syndrome, and is associated with functional impairment of vascular smooth muscle (VSM) cells. Sirtuin1 (SirT1) is a NAD+-dependent deacetylase induced in response to cellular stresses. Our goal was to study the effects of VSM SirT1 on AS in the context of diet-induced metabolic syndrome.

VSM specific genetic SirT1 over-expression (SMTG) prevented AS induced by HFHS, measured in vivo by pulse wave velocity (PWV) over 8 months. Resveratrol or S17834, two polyphenolic compounds known to activate SirT1, prevented HFHS-induced AS and were mimicked by global SirT1 over-expression (SirBACO), without evident metabolic improvements (HFHS-induced weight gains or response to a glucose tolerance test remained unchanged). Additionally, HFHS-induced PWV increases were reversed by one-week treatment with a specific, small molecule SirT1 activator (SRT1720). Overnight fasting acutely decreased PWV in mice fed HFHS for 8 months, but not in mice lacking SirT1 in VSM (SMKO). These beneficial effects of pharmacological, genetic or fasting-induced SirT1 activation against AS, were associated with a decrease in NFκB activation and VCAM-1 and p47phox protein expressions, in aorta and VSM cells. In conclusion, VSM SirT1 activation decreases AS in the setting of obesity by stimulating anti-inflammatory and anti-oxidant pathways in the aortic wall. SirT1 activators may represent a novel therapeutic approach to prevent AS and associated CV complications in overweight/obese individuals with metabolic syndrome.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PWV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT/HFHS (n=5)</td>
<td>5.18 ± 0.73</td>
</tr>
<tr>
<td>SMTG/HFHS (n=6)</td>
<td>2.55 ± 0.32 †</td>
</tr>
<tr>
<td>WT/HFHS/Resveratrol (n=6)</td>
<td>3.67 ± 0.52 †</td>
</tr>
<tr>
<td>WT/HFHS/S17834 (n=6)</td>
<td>3.00 ± 0.32 †</td>
</tr>
<tr>
<td>SirBACO/HFHS (n=5)</td>
<td>2.43 ± 0.22 †</td>
</tr>
<tr>
<td>WT/HFHS/SRT1720 (n=5)</td>
<td>2.98 ± 0.27 †</td>
</tr>
<tr>
<td>WT/HFHS/Fed (n=9)</td>
<td>4.74 ± 0.57</td>
</tr>
<tr>
<td>WT/HFHS/Fasted (n=9)</td>
<td>2.32 ± 0.15 *</td>
</tr>
<tr>
<td>SMKO/HFHS/Fed (n=5)</td>
<td>3.98 ± 0.64</td>
</tr>
<tr>
<td>SMKO/HFHS/Fasted (n=5)</td>
<td>3.99 ± 0.14</td>
</tr>
</tbody>
</table>

SMTG, VSM SirT1 overexpressing mice
SirBACO, whole body SirT1 overexpressing mice
SMKO, VSM SirT1 knock out mice
HFHS, high fat, high sucrose diet
†, p<0.05 vs WT/HFHS
*, p< 0.05 vs WT/HFHS/Fed

J. Fry: None. L. Al Sayah: None. R. Weisbrod: None. I. Van Roy: None. X. Weng: None. R. Cohen: None. M. Bachschmid: None. F. Seta: None.

Funding: No
Funding Component: 111

Xanthine Oxidoreductase Plays a Key Role in Nitrate, Nitrite and Nitric Oxide Homeostasis When the Endothelial Nitric Oxide Synthase is Compromised

Maria Peleli, Christa Zollbrecht, Marcelo Montenegro, Michael Hezel, Eddie Weitzberg, Jon O Lundberg, Mattias Carlström, Karolinska Instt, Solna, Stockholm, Sweden
Xanthine oxidoreductase (XOR) is generally known as a source of superoxide production, but this enzyme has also been suggested to mediate NO production via reduction of inorganic nitrate (NO$_3^-$) and nitrite (NO$_2^-$). This pathway for NO generation is of particular importance during certain pathologies, whereas endothelial NO synthase (eNOS) is the primary source of vascular NO generation under normal physiological conditions. The exact interplay between the NOS and XOR-derived NO is not yet fully elucidated. The aim of the present study was to investigate if eNOS deficiency is partly compensated by XOR upregulation and sensitization of the NO$_3^-$ - NO$_2^-$ - NO pathway. NO$_3^-$ and NO$_2^-$ were similar between naïve eNOS KO and wildtype (wt) mice, but reduced upon chronic treatment with the non-selective NOS inhibitor L-NAME (wt: 25.0±5.2, eNOS KO: 39.2±6.4, L-NAME: 8.2±1.6 μM NO$_3^-$, wt: 0.38±0.07, eNOS KO: 0.42±0.04, L-NAME: 0.12±0.02 μM NO$_2^-$ ). XOR function was upregulated in eNOS KO compared with wt mice [(mRNA: wt 1±0.07, eNOS KO 1.38±0.17), (activity: wt 825±54, eNOS KO 1327±280 CLU/mg/min), (uric acid: wt 32.87±1.53, eNOS KO 43.23±3.54 μM)]. None of these markers of XOR activity was increased in nNOS KO and iNOS KO mice. Following acute dose of NO$_3^-$ (10 mg/kg bw, i.p.), the increase of plasma NO$_2^-$ was more pronounced in eNOS KO (+0.51±0.13 μM) compared with wt (+0.22±0.09 μM), and this augmented response in the eNOS KO was abolished by treatment with the highly selective XOR inhibitor febuxostat (FEB). Liver from eNOS KO had higher reducing capacity of NO$_2^-$ to NO compared with wt, and this effect was attenuated by FEB (Δppb of NO: wt +8.7±4.2, eNOS KO +44.2±15.0, wt+FEB +22.2±9.6, eNOS KO+FEB +26.8±10.2). Treatment with FEB increased blood pressure in eNOS KO (ΔMAP: +10.2±5.6 mmHg), but had no effect in wt (ΔMAP: -0.6±3.3 mmHg). Supplementation with NO$_3^-$ (10 mM, drinking water) reduced blood pressure in eNOS KO (ΔMAP: -6.3±2.2 mmHg), and this effect was abolished by FEB (ΔMAP: +1.1±1.9 mmHg). In conclusion, upregulated and altered XOR function in conditions with eNOS deficiency can facilitate the NO$_3^-$ - NO$_2^-$ - NO pathway and hence play a significant role in vascular NO homeostasis.


Funding: No

Funding Component: 112

The 20-HETE Receptor: a New Player in Hypertension

Victor Garcia, Ankit Gilani, Brian Shkolnik, New York Medical Coll, Valhalla, NY; John R Falck, Univ of Texas Southwestern, Dallas, TX; Varun Pandey, New York Medical Coll, Valhalla, NY; Jorge H Capdevila, Vanderbilt Univ, Nashville, TN; Michal L Schwartzman, New York Medical Coll, Valhalla, NY

Here, we report that GPR75, a G protein-coupled receptor of the Gq rhodopsin subfamily, selectively binds 20-hydroxyeicosatetraenoic acid (20-HETE), a cytochrome P450-derived bioactive arachidonic acid metabolite implicated in the pathogenesis of hypertension and cardiovascular diseases. In endothelial cells, 20-HETE binding to GPR75 stimulates β-arrestin recruitment and GIT1-GPR75 association, which further facilitates a c-Src-mediated transactivation of EGFR. This results in downstream signaling pathways which induce ACE expression and decrease NO bioavailability. Knockdown of GPR75 prevents 20-HETE-mediated downstream effects in endothelial cells including EGFR activation and ACE induction. In vascular smooth muscle cells, GPR75-20-HETE pairing is associated with Ga$_{q/11}$-and GIT1-mediated PKC-stimulated
phosphorylation of MaxiKβ, linking GPR75 activation to 20-HETE-mediated vasoconstriction. We used the conditional Cyp4a12tg mice, which display doxycycline (DOX)-mediated hypertension along with vascular dysfunction and remodeling in a 20-HETE-dependent manner, to assess whether GPR75 is a necessary component of 20-HETE pro-hypertensive actions. Administration of GPR75-targeted shRNA lentiviral particles to DOX-treated Cyp4a12tg mice, which resulted in 80% knockdown of GPR75 knockdown, prevented blood pressure elevation (100±3 vs 135±2 mmHg) and 20-HETE-mediated increases in ACE expression, endothelial dysfunction, smooth muscle contractility and vascular remodeling when compared to DOX-treated Cyp4a12tg mice receiving non-targeted shRNA. The discovery of 20-HETE-GPR75 pairing provides the molecular basis for the signaling and pathophysiological bioactions mediated by 20-HETE in hypertension. These results clearly place GPR75 as a novel target in the control of blood pressure and vascular function.


Funding: Yes
Funding Component: Founders Affiliate (Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island, Vermont)

113

Ambulatory Blood Pressure in Hypertensive Patients with Inclusion Criteria for the SPRINT Trial

Alejandro De La Sierra, Hosp Mutua Terrassa, Terrassa, Spain; Jose R Banegas MD, Univ Autonoma, Madrid, Spain; Juan A Divison, Cap Casas Ibañez, Albacete, Spain; Manuel Gorostidi, Hosp Central Asturias, Oviedo, Spain; Ernest Vinyoles, Cap La Mina, Barcelona, Spain; Juan J De La Cruz, Univ Autonoma, Madrid, Spain; Julian Segura, Luis M Ruilope, Hosp 12 De Octubre, Madrid, Spain

The SPRINT trial has demonstrated the benefit of intensive BP reduction in hypertensive patients at high cardiovascular risk. Values of ABPM are of potential interest in such patients to better select those who will benefit of a lower BP target. We aimed to evaluate ABPM values in a large cohort of patients potentially candidates (meeting inclusion criteria) for the SPRINT trial. Moreover, in patients on antihypertensive therapy who also fulfill SPRINT criteria, except for clinic SBP ≥ 130 mmHg, we evaluated 24-hour SBP values among those who had clinic SBP < 120; between 120 and 139, or ≥ 140. From the database of the Spanish ABPM Registry containing 115708 patients, we identified 39132 (34%, 51% women, mean age 65 years) who fulfill both inclusion and exclusion criteria of the SPRINT trial. Mean values of clinic SBP were 151±11 mmHg, whereas corresponding values for 24-h SBP were 130±13 mmHg. Overall, 52% of patients had 24-h SBP below 130 mmHg. The proportion varied from 69% in those with clinic BP 130-139 to 34% in those with clinic BP ≥ 170 mmHg. Among 34328 treated patients who fulfilled SPRINT inclusion criteria (except for clinic BP ≥ 130), 1014 (3%) had clinic SBP < 120 mmHg, and 5330 (16%) values between 120 and 139 mmHg. The remaining 27984 patients were not controlled and had clinic SBP ≥ 140 mmHg. Values of 24 h SBP below 130 mmHg were seen in 88% of those with clinic BP < 120, in 74% of those with clinic BP 120-139, and in 47% of those with clinic BP ≥ 140 mmHg. The corresponding proportion of patients having 24-h SBP < 100 mmHg were 7.7%, 1.1%, and 0.6%. We conclude that ABPM assessment could be necessary in the evaluation of hypertensive patients at high CV risk before targeting the BP
goal, as roughly half of them may have normal values of 24-h SBP (< 130 mmHg). In addition, targeting clinic BP below 120 mmHg is accompanied by 8% of patients with 24-h SBP below 100 mmHg.


Funding: No

Funding Component: 114

Renal Denervation Reduces Monocyte Activation: An Anti-inflammatory Effect Relevant for Cardiovascular Risk

Maria Teresa Kristina Zaldivia, Jennifer Rivera, Baker IDI Heart and Diabetes Inst, Melbourne, Australia; Dagmara Hering, Petra Marusic, Petra Marusic, Dobney Hypertension Ctr, Sch of Med and Pharmacology- Royal Perth Hosp Unit, Univ of Western Australia, Perth, Australia; Yusuke Sata, Nina Eikelis, Rebecca Lee, Gavin W. Lambert, Nay M. Htun, Jacqueline Duval, Louise Hammond, Steffen Eisenhardt, Ulrike Flierl, Baker IDI Heart and Diabetes Inst, Melbourne, Australia; Markus Schlaich, Dobney Hypertension Ctr, Sch of Med and Pharmacology- Royal Perth Hosp Unit, Univ of Western Australia, Perth, Australia; Karlheinz Peter, Baker IDI Heart and Diabetes Inst, Melbourne, Australia

Background Over-activation of renal sympathetic nervous system and low-grade systemic inflammation are thought to be common features of hypertension. Renal Denervation (RDN) reduces sympathetic activity in patients with resistant hypertension. However, its effect on systemic inflammation has not been investigated. Aim To determine the effect of RDN-induced sympathetic inhibition on monocyte activation and systemic inflammation in hypertensive patients. Methods Peripheral blood was obtained from 42 patients who underwent RDN for uncontrolled blood pressure (BP) at baseline, at 3 months and 6 months post-procedure. Ambulatory BP, overall activation status of monocyte as well as monocyte subsets and inflammatory markers were assessed at each time point. Results RDN significantly lowered 24-hour ambulatory BP at 3 months (150.5/81.0 mmHg to 144.7/77.9 mmHg), which was sustained at 6 months (144.7/78.6 mmHg). The overall monocyte activation was significantly decreased (3 months, 4079.4 MFI to 3182.0 MFI; 6 months, 3457.62 MFI) post-RDN, specifically in the subset of classical monocytes (6 months, 4696.8 MFI to 3958.8 MFI). In line with this, reduction of several inflammatory markers were observed, including monocyte-platelet aggregates at 3 months (34% [680 of 2000 monocyte events] to 11.85% [237 of 2000 monocyte events]) and plasma levels of MCP-1 (3 months, 144.9 pg/ml to 100.1 pg/ml; 6 months, 122.2 pg/ml), IL-1β (3 months, 18.3 pg/ml to 10.8 pg/ml; 6 months, 12.2 pg/ml), TNF-α (3 months, 167.5 pg/ml to 78.4 pg/ml; 6 months, 111.1 pg/ml), IL-12 (3 months, 59.8 pg/ml to 9.9 pg/ml; 6 months, 21.4 pg/ml) and IL-6 (3 months, 2.4 pg/ml to 1.5 pg/ml; 6 months, 1.9 pg/ml). A positive correlation was observed between baseline muscle sympathetic nerve activity and monocyte activation (R=0.62) and changes observed at both time points (3 months, R=0.63; 6 months, R=0.88) post-procedure. Conclusions Inhibition of sympathetic activity via RDN is associated with a reduction of monocyte activation and other circulating inflammatory markers in hypertensive patients. These findings point to a direct interaction between the inflammatory and sympathetic nervous system, which is of central relevance for the understanding of beneficial cardiovascular effects of RDN.
We studied the relationship of blood pressure (BP) trajectories during the first seven days after symptom onset with short- and long-term major clinical outcomes among patients with acute ischemic stroke. A total of 4,036 patients with acute ischemic stroke and elevated systolic BP from the CATIS trial were included in this analysis. Three BPs were measured every 2 hours for the first 24 hours, every 4 hours during the second and third days, and every 8 hours thereafter for the remainder of the seven days. Latent class models were used to identify subgroups that share a similar underlying trajectory in BP in the acute phase. Five systolic BP trajectories of high, high-to-moderate low, moderate high, moderate low, and low were identified. Compared to the high trajectory, multiple-adjusted odds ratios (95% CI) of death and major disability at 3 months for high-to-moderate low, moderate high, moderate low, and low trajectories were 0.61 (0.44 to 0.86), 0.63 (0.48 to 0.84), 0.49 (0.37 to 0.65), and 0.42 (0.30 to 0.59), respectively (overall p<0.0001). Likewise, the corresponding multiple-adjusted odds ratios at 2 years were 0.64 (0.46 to 0.90), 0.78 (0.59 to 1.04), 0.49 (0.37 to 0.66), and 0.49 (0.34 to 0.69), respectively (overall p<0.0001). These data indicate that individuals with a consistently high systolic BP during the acute phase of ischemic stroke had the highest risk of short- and long-term death and major disability. In addition, moderate systolic BP reduction to below 140 mmHg from higher levels lowers risk of short- and long-term death and major disability.
The Effect of a Practice-based Multi-component Intervention That Includes Health Coaching on Medication Adherence and Blood Pressure Control in Rural Primary Care

Jia-Rong Wu, Univ of North Carolina-Chapel Hill, Chapel Hill, NC; Doyle M Cummings, East Carolina Univ, Greenville, NC; Quefeng Li, Jacquie Halladay, Katrina Donahue, Crystal Cene, Alan Hinderliter, Univ of North Carolina-Chapel Hill, Chapel Hill, NC; Hayden Bosworth, Duke Univ, Durham, NC; Cassandra Miller, Beverly Garcia, Univ of North Carolina-Chapel Hill, Chapel Hill, NC; Jim Tillman, East Carolina Univ, Greenville, NC; Darren DeWalt, Univ of North Carolina-Chapel Hill, Chapel Hill, NC

Background: Lower adherence to anti-hypertensive medications contributes to sub-optimal patient outcomes, yet there are few successful interventions in rural primary care that target improved adherence. The purpose of this study was to determine whether a multi-component quality improvement intervention that included literacy-sensitive health coaching with motivational interviewing was associated with improved medication adherence and reductions in blood pressure (BP) in patients with a history of uncontrolled hypertension (HTN).

Methods: Adult patients in six rural primary care settings with one or more visits in the last year with a systolic BP > 150 mmHg were recruited. Project faculty facilitated systematic changes in care delivery in local practices. Patients also received monthly phone-based literacy-sensitive health coaching including a focus on medication adherence, and a BP cuff for home monitoring. Data regarding medication adherence (Morisky Medication Adherence Scale-8) and BP were collected at baseline, 6, 12, 18, and 24 months. Linear mixed effects modeling was used to determine the effects of the multi-component intervention on medication adherence and whether changes in medication adherence were associated with changes in systolic and diastolic BP.

Results: There were 477 patients enrolled; the majority were female, black, and reported an annual household income of < $40,000. At baseline, 39% of the patients had low medication adherence (MMAS-8 score < 6). In linear mixed effects models, the intervention resulted in modest increases in medication adherence [5.75 ± 1.37 at baseline to 5.94 ± 1.33 at 24 months (p = .04)]. Corresponding changes in BP were: from 138.6 ± 21.8/81.6 ± 12.9 mmHg at baseline to 132.7 ± 19.5/76.1 ± 14.5 mmHg at 24 months follow-up [mean 0.22-0.25/0.24-0.26 mmHg per month before and after adjustment for covariates (p < .001)]. Changes in medication adherence were significantly associated with reductions in diastolic BP longitudinally (p = .047).

Conclusion: A practice-based quality improvement intervention that includes health coaching is associated with improvements in medication adherence and BP, and offers promise as a clinically applicable intervention in rural primary care.

Preclinical Target Organ Damage Changes in Patients with Resistant Hypertension Randomized to Renal Denervation or
Spironolactone as Add-on Therapy. Results From the DENERVHTA Study

Anna Oliveras, Hosp del Mar, Barcelona, Spain; Pedro Armario, Moisés Broggi, Consorci Sanitari Integral, Univ of Barcelona, Sant Joan Despí, Spain; Laia Sans, Albert Clarà, Susana Vázquez, Lluis Molina, Hosp del Mar, Barcelona, Spain; Júlia Pareja, Hosp Mútua de Terrassa, Terrassa, Spain; Julio Pascual, Hosp del Mar, Barcelona, Spain; Alejandro de la Sierra, Hosp Mútua de Terrassa, Terrassa, Spain

Objective
To compare the effect of renal denervation (RDN) and spironolactone, two proposed therapeutic strategies for the treatment of patients with resistant hypertension (RH), on preclinical target organ damage (pTOD).

Methods
Patients with office systolic blood pressure (SBP) ≥150 mmHg and 24h-SBP ≥140 mmHg despite receiving ≥3 full-dose antihypertensive drugs, one a diuretic, but none aldosterone antagonist, were randomized to receive RDN or spironolactone (50mg), as add-on therapy. Changes (Δ) in 24h-BP, as well as Δ in urinary albumin excretion (ΔUAE), carotid-femoral pulse-wave velocity (ΔcfPWV), carotid intima-media thickness (ΔIMT), left ventricular mass index (ΔLVMI) and E/e’ (ΔE/e’), a marker of hypertension-induced diastolic dysfunction, were evaluated at 6 months. Between-group comparisons of ΔUAE (after log transformation), ΔcfPWV, ΔIMT, ΔLVMI and ΔE/e’ were carried out by generalized linear models before and after adjusting by Δ24h-SBP and the corresponding baseline value.

Results
Twenty-four patients (mean age 64±8 yr) were included. Mean baseline-adjusted difference (95% CI) between the two groups (Spironolactone vs. RDN) at 6 months in 24h-SBP (mmHg) was of -17.9 (-30.9 to -4.9), p=0.01.

Conclusion
Changes at 6 months on pTOD as assessed by UAE, cfPWV, IMT, LVMI and E/e’, were not associated with the therapeutic add-on strategy used to reduce high blood pressure in RH patients. We cannot discard that the high variability of some of these markers, especially UAE, could account for this lack of statistically significant between-group differences.

As shown in the table, there were no statistically significant between-group differences in Δ on pTOD.

<table>
<thead>
<tr>
<th></th>
<th>Spironolactone (n=13)</th>
<th>Renal denervation (n=11)</th>
<th>p-value</th>
<th>adjusted* p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVMI (µg/dl)</td>
<td>Change at 6 months</td>
<td>Change at 6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UAE (µg/dl)</td>
<td>-5.41 (-32.0; 12.2)</td>
<td>-1.83 (-16.6; 20.2)</td>
<td>0.561</td>
<td>0.405</td>
</tr>
<tr>
<td>E/e’ value</td>
<td>-0.61 (-2.5; 2.0)</td>
<td>-0.32 (-2.0; 2.58)</td>
<td>0.795</td>
<td>0.594</td>
</tr>
<tr>
<td>carotidIMT (mm)</td>
<td>-0.03 (-0.13; 0.05)</td>
<td>-0.29 (-0.05; 1.29)</td>
<td>0.386</td>
<td>0.720</td>
</tr>
<tr>
<td>cfPWV (m/s)</td>
<td>-1.14 (-2.15; -0.15)</td>
<td>-0.69 (1.53; 0.15)</td>
<td>0.259</td>
<td>0.477</td>
</tr>
<tr>
<td>LVMI (mg/ml)</td>
<td>-87.7 (-164.5; -9.9)</td>
<td>-23.8 (104.9; 56.9)</td>
<td>0.042</td>
<td>0.695</td>
</tr>
</tbody>
</table>

* Adjusted by respective baseline value and Δ of 24h-SBP

Funding: No

Nebivolol Therapy Enhances Endothelial Fibrinolytic Capacity in Adults with Elevated Blood Pressure

Christopher A DeSouza, Tyler D Bammert, Kyle J Diehl, Caitlin A Dow, Jared J Greiner, Univ of Colorado Boulder, Boulder, CO; Brian L Stauffer, Univ of Colorado Sch of Med, Aurora, CO
Impaired endothelial fibrinolytic function contributes to increased thrombotic risk with elevated blood pressure. *In vitro* data suggests that the antihypertensive, nebivolol (N), favorably effects the fibrinolytic system, but there is no *in vivo* clinical evidence that N treatment improves endothelial fibrinolytic function. We hypothesized that chronic N therapy will increase the capacity of the endothelium to release tissue-type plasminogen activator (t-PA) in adults with elevated blood pressure (BP ≥ 130/85 mm Hg). In an ongoing study, 36 middle-aged adults (age: 44-67 years) were treated for 12 weeks: 12 with N (5 mg/d; BP 141/86±2/2 mmHg); 12 with metoprolol succinate (M: 100 mg/d; 140/90±3/2 mmHg); and 12 with placebo (P; 138/85±2/2 mmHg). Before and after intervention, net endothelial release of t-PA was determined, *in vivo*, in response to intrabrachial infusions of bradykinin (BK: 125-500 ng/min) and sodium nitroprusside (SNP: 2-8 µg/min). Subject characteristics (age, BMI, systolic and diastolic BP) were similar between the groups. Blood pressure was lowered (P< 0.05) to a similar extent by both N (124/78±3/2 mmHg) and M (124/78±3/1 mmHg) but unchanged by P (134/80±3/2 mmHg). Endothelial t-PA release in response to BK was not significantly different between the N (-2.8±1.4 to 49.2±5.6 ng/100 mL tissue/min), M (-0.5±1.3 to 53.5±8.5 ng/100 mL tissue/min) and P (0.1±1.1 to 52.0±5.6 ng/100 mL tissue/min) groups prior to intervention. After intervention, only N therapy affected t-PA release; the capacity of the endothelium to release t-PA in the N group was significantly higher (-1.9±1.6 to 72.6±7.1 ng/100 mL tissue/min; P< 0.05). Total amount of t-PA released (area under the BK curve) markedly increased (45%) in response to N (308±39 vs 445±47 ng/100 mL tissue; P< 0.05). In contrast, endothelial t-PA release was not significantly altered by M (-1.6±1.1 to 54.0±6.1 ng/100 mL tissue/min). As expected, t-PA release was unchanged with P. These results demonstrate that, in spite of similar reductions in blood pressure, N, but not M, treatment increases the capacity of the endothelium to release t-PA in adults with elevated blood pressure. Enhanced endothelial fibrinolytic function with N may provide an important vascular benefit in this at risk population.

**C.A. DeSouza:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Forest Laboratories, Inc.  
**T.D. Bammert:** None.  
**K.J. Diehl:** None.  
**C.A. Dow:** None.  
**B.L. Stauffer:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Forest Laboratories, Inc.

Funding: No  
Funding Component: 119

**Anti-hypertensive Effect of Thiazides Shifts From Salt Excretion to Vasorelaxation During Salt Restriction or Volume Depletion**

*Saeed Alshahrani*, Kamyar Zahedi, Min Jiang, Michelle Nieman, Sharon Barone, John Lorenz, Jack Rubinstein, Manoocher Soleimani, Univ of Cincinnati, Cincinnati, OH

Thiazide derivatives, including hydrochlorothiazide (HCTZ), are specific inhibitors of the Na⁺-Cl⁻ Co-transporter (NCC) in the kidney distal tubules, and the most commonly used diuretic for the treatment of mild to moderate hypertension. Both renal (natriuretic) and extra renal (vasorelaxation) mechanisms have been proposed as major mediators of blood pressure reduction by HCTZ but the circumstances under which the renal or extra renal mechanism predominates remain unknown. To address these issues, systemic
blood pressure was monitored by intra-arterial catheter and computerized tail cuff in transgenic mice lacking NCC under varying conditions. For comparison, mice with pendrin ablation or double deletion of pendrin and NCC were used to ascertain the compensatory role of pendrin in salt reabsorption in response to HCTZ. Pendrin KO mice were the only group which showed enhanced salt excretion in response to HCTZ, with salt excretion increasing by ~30% in pendrin KO vs. WT mice (p<0.05). In mice lacking NCC, HCTZ significantly reduced the systemic blood pressure only during salt restriction and without enhancing salt excretion. In volume depleted but not in volume resuscitated NCC/pendrin dKO mice, HCTZ caused dramatic reduction in systemic blood pressure, with systolic blood pressure decreasing from 72.13± 5.1 at baseline to 51.06 ± 6.6 mm Hg in dKO mice within 20 minutes of HCTZ administration (p<0.01 vs. baseline) with no significant effect in WT mice (p>0.5 vs. baseline) or in salt resuscitated NCC/pendrin dKO mice. There was no enhancement in salt excretion and no reduction in echocardiography-monitored cardiac output in pendrin/NCC dKO mice in response to HCTZ. The antihypertensive effects of HCTZ were abrogated in the presence of paxilline, a specific blocker of BK channel, which is upregulated in arterial vasculature of volume depleted mutant mice. We propose that thiazides reduce blood pressure predominantly via vasorelaxation during salt restriction/volume depletion; whereas, they enhance salt excretion during salt replete state and specifically in conditions associated with pendrin downregulation/inactivation.


Funding: No

Reversal of Hypertension Stops, but Does Not Reverse, Renal T-cell Infiltration in the Dahl Salt-sensitive Rat

Louise C Evans, Galina Petrova, Theresa Kurth, David L Mattson, Allen W Cowley Jr., Medical Coll of Wisconsin, Milwaukee, WI

In previous chronic servo control experiments we showed that increased renal perfusion pressure (RPP) amplifies renal T-cell infiltration in Dahl salt-sensitive (SS) rats. Renal T-cell infiltration was significantly higher in the right-hypertensive kidneys vs. the left servo-controlled kidneys of SS rats fed high salt (HS) for 7-days. There was no significant difference in T-cell infiltration in sham rats (both kidneys exposed to comparable RPPs). Here we use the chronic servo control system to determine whether the reversal of salt-sensitive hypertension reverses renal T-cell infiltration in SS rats. An aortic balloon occluder was placed around the aorta, between the renal arteries to electronically servo-control RPP to the left kidney. Arterial catheters were used to measure MAP above and below the occluder (n=5). Baseline RPPs were measured on a control salt diet (0.4% NaCl) for 3-days (average left RPP, 124±1mmHg) after which rats were switched to a HS diet (4.0% NaCl) for 14-days. During the first 7-days of HS both kidneys were exposed to uncontrolled hypertension. After 7-days a servo control system was used to bring the RPP to the left kidney (the reversal kidney) back to the average control level, where it was held for the next 7-days of HS (from 139±2mmHg to 125±2mmHg). It was found that reversal of hypertension prevented a further increase of renal T-cell infiltration but the number of infiltrated T-cells did not diminish. Moreover, the number of T-cells in the left-reversal kidney was significantly higher than in the

Funding Component: 120

In previous chronic servo control experiments we showed that increased renal perfusion pressure (RPP) amplifies renal T-cell infiltration in Dahl salt-sensitive (SS) rats. Renal T-cell infiltration was significantly higher in the right-hypertensive kidneys vs. the left servo-controlled kidneys of SS rats fed high salt (HS) for 7-days. There was no significant difference in T-cell infiltration in sham rats (both kidneys exposed to comparable RPPs). Here we use the chronic servo control system to determine whether the reversal of salt-sensitive hypertension reverses renal T-cell infiltration in SS rats. An aortic balloon occluder was placed around the aorta, between the renal arteries to electronically servo-control RPP to the left kidney. Arterial catheters were used to measure MAP above and below the occluder (n=5). Baseline RPPs were measured on a control salt diet (0.4% NaCl) for 3-days (average left RPP, 124±1mmHg) after which rats were switched to a HS diet (4.0% NaCl) for 14-days. During the first 7-days of HS both kidneys were exposed to uncontrolled hypertension. After 7-days a servo control system was used to bring the RPP to the left kidney (the reversal kidney) back to the average control level, where it was held for the next 7-days of HS (from 139±2mmHg to 125±2mmHg). It was found that reversal of hypertension prevented a further increase of renal T-cell infiltration but the number of infiltrated T-cells did not diminish. Moreover, the number of T-cells in the left-reversal kidney was significantly higher than in the
continuously-controlled, left-servo kidney despite comparable final RPPs (129±2mmHg to the left-servo kidney vs. 125±2mmHg to the left-reversal kidney). T-cell infiltration in the left-reversal kidney was comparable to the left-sham kidneys, in which the final RPP was 148±2mmHg.

In ‘T-cells per kidney’ -
CD3+ (mature): Reversal=287x10^3±65x10^3 Servo=78x10^3±12x10^3 Sham=186x10^3±47x10^3.
CD3+CD4+ (helper): Reversal=95x10^3±27x10^3 Servo=32x10^3±5x10^3 Sham=86x10^3±26x10^3.
CD3+CD8+ (cytotoxic):
Reversal=196x10^3±42x10^3 Servo=42x10^3±7x10^3 Sham=102,082x10^3±23x10^3.

We conclude that reversal of blood pressure alone, for 1-week, while HS intake is continued, stops but does not reverse renal T-cell infiltration in SS rats.

Funding: No
Funding Component: 121

Afferent Renal Nerve Chemo- and Mechanosensitive Responses and the Modulation of Sodium Homeostasis and Blood Pressure

Alissa A Frame, Casey Y Carmichael, Kathryn R Walsh, Richard D Wainford, Boston Univ, Boston, MA

Aim: We hypothesize that challenges to sodium homeostasis differentially activate chemo- vs. mechanosensitive afferent renal nerves to evoke sympathoinhibition, sodium homeostasis and normotension in the Sprague-Dawley (SD) rat.

Methods: Conscious SD rats, post sham (S) or afferent renal nerve ablation (Renal-CAP; capsaicin 33 mM) underwent IV volume expansion (VE; 5% BW) or IV sodium loading (1M NaCl Infusion – constant infusion volume) and HR, MAP, natriuresis and PVN neuronal activation (c-Fos expression) were assessed (N=4/gp). Naive SD rats were fed a 0.6% (NS) or 4% NaCl (HS) diet for 21 days and afferent renal nerve activity was assessed as norepinephrine (NE) (1250 pmol) and NaCl-evoked (450mM) substance P (SP) release in a renal pelvic assay (N=4/gp). Radiotelemetered SD rats post S or Renal-CAP immediately prior a 0.6% (NS) or 4% NaCl (HS) diet underwent continuous MAP monitoring. On day-21 plasma and renal NE content was assessed (N=5/group).

Results: Renal-CAP attenuated the natriuretic and PVN parvocellular responses to IV VE (peak UNaV [μeq/min]; S 43±4 vs Renal-CAP 26±6, P<0.05, PVN Medial Parvocellular neuronal activation [c-fos positive cells]; S 49±6 vs Renal-CAP 22±5 P<0.05) and evoked increased MAP (MAP 90min post-VE [mmHg] S 118±3 vs Renal-CAP 132±4, P<0.05). In contrast Renal-CAP did not alter the natriuresis to IV 1M NaCl (UNaV [μeq/min]; S 21±4 vs Renal-CAP 21±3) or increase MAP. In naïve SD rats HS-intake did not alter MAP and suppressed plasma and renal NE (P<0.05). HS intake increased NE, but not NaCl, mediated afferent renal nerve activity (NE-evoked peak ΔSP [ng/ml]; NS 14±2, HS 22±3, P<0.05, NaCl-evoked peak ΔSP [ng/ml]; NS 17±3, HS 16±2). Renal-CAP immediately prior to a HS-intake persistently increased MAP (Day 21 MAP [mmHg] S HS 106±4, Renal-CAP HS 123±5, P<0.05) and attenuated HS-evoked global and renal sympathoinhibition (P<0.05).

Conclusion: The mechanosensitive afferent renal nerves mediate acute natriuresis and blood pressure regulation via activation of PVN sympathoinhibitory neurons. During HS intake the afferent renal nerves counter the development of salt-sensitive hypertension via a mechanism involving increased mechano but not chemosensitive afferent nerve responsiveness to potentiate sympathoinhibition.
We hypothesized that tumor necrosis factor-alpha (TNF) derived from the thick ascending limb (TAL) modulates renal adaptation to high NaCl (HS) intake. TNF levels were higher in urine (40±4 vs 18±5 pg/24 h), but not plasma (20±3 vs 18±3 pg/ml), of mice that ingested 1% NaCl in the drinking water compared with tap water. HS intake increased TNF mRNA abundance in mTAL tubules by more than 4-fold (p<0.05). Intrarenal injection of a lentivirus construct that silences TNF in the kidney, EGFP-TNF-ex4, decreased renal TNF mRNA levels induced by HS intake by more than 80% (p<0.05). Injection of control (U6) or TNF silencing lentivirus had no effect on baseline blood pressure in mice ingesting a NSD and tap water. However, renal-specific silencing of TNF rapidly increased blood pressure in mice drinking 1% NaCl (U6: 110±4 vs shTNF: 136±6 mmHg; p<0.05), an effect that was sustained for 11 days. Blood pressure returned to baseline after mice were switched to tap water (days 12-16), and rapidly increased when they were switched back to HS (days 17-21). Accumulation of NKCC2A mRNA in renal outer medulla increased approximately 3-fold (p<0.05) after injection of EGFP-TNF-ex4 followed by ingestion of HS for 3 days compared with U6 control groups. Generation of TAL-specific TNF knockout mice (TAL-TNF KO) was accomplished using the Cre/loxP recombination system. Specific recombination in the TAL was validated by PCR of microdissected nephron segments. The recombined allele product (0.45kb) was present in TAL tubules but not PT, IMCD, or spleen. Baseline blood pressure was similar in TNF-TAL KO mice and littermate controls but increased when TNF-TAL KO mice were given HS for three days. Food, water intake, and body weight were not different between groups in either model of TNF deletion. Moreover, the increases in blood pressure were not associated with damage to glomeruli, blood vessels, or tubules; no infiltration of inflammatory cells was observed. The increase in renal TNF levels in response to HS intake without a concomitant increase in blood pressure, and the increase in blood pressure in response to HS when TNF is silenced in the TAL suggests deficiency of TAL-derived TNF is sufficient to render normotensive healthy mice salt-sensitive in the absence of ongoing inflammation.
A Fructose-enriched Diet Stimulates Superoxide Production in Juxtaglomerular Cells and Prevents High-salt Induced Inhibition of Plasma Renin Activity (PRA)

Mariela Mendez, Kevin L Gordish, Emily Henson, Pablo A. Ortiz, William H Beierwaltes, Henry Ford Hosp, Detroit, MI

A fructose-enriched diet has been associated with hypertension. Western diets are rich in fructose and salt. We found that a fructose enriched diet plus high-salt induced salt sensitive hypertension. Plasma renin activity (PRA) is essential for blood pressure (BP) control. A high-salt diet decreases PRA by inhibiting renin release from juxtaglomerular (JG) cells. However it is not known if dietary fructose might impair the inhibition of renin release by high salt to promote salt sensitivity. Salt sensitive rats have enhanced levels of superoxide in the renal cortex, and we found that superoxide stimulates renin release from JG cells. Thus, we hypothesized that a fructose-enriched diet (20%) promotes salt sensitive hypertension in part by preventing high salt-induced inhibition of renin release from JG cells by enhancing superoxide production. To test this, Sprague Dawley rats were given 20% fructose in their drinking water, with normal or high salt diet (4% NaCl) for up to 4 weeks. Feeding normal rats a fructose+High-salt diet increased systolic BP by 30 mmHg whereas fructose or high-salt alone did not change BP (High-salt = 125±4, Fruct = 131±4, Fruct+High-salt = 147±7; n=6, p<0.05). A high-salt diet alone for 4 weeks decreased PRA by 85%. However, in rats fed fructose+High-salt diet did not decrease PRA (in ng All/ml/hr: Ctrl = 2.51±0.72, High-salt = 0.43±0.07, Fruct = 2.71±0.9, Fruct+High-salt = 1.89±0.43; n=10, p<0.05). We next examined the role of the fructose or fructose+High-salt diet on NADPH oxidase expression in isolated JG cells. NOX4 expression was enhanced in JG cells from rats fed fructose+High-salt diet (n=4; p<0.05). Next, we measured superoxide production with Dihydroethidium and found it was higher in JG cells from rats fed fructose+High-salt diet compared to high-salt alone (% of Ctrl: High-salt = 90.7±37; fruct+High-salt = 289±85; n=4; p<0.05). We conclude that a 20% fructose-diet promotes salt sensitivity of BP. The mechanism may involve enhanced NOX4 expression and elevated superoxide levels within JG cells stimulating renin release. 15 million Americans consume 20% of their calories from fructose, and most, 4-8 times the recommended salt intake. Decreasing fructose intake could have a beneficial BP effect in hypertensive patients.


Funding: Yes
Funding Component: National Center

Soluble (Pro)Renin Receptor Regulation of ENaC in Angiotensin II Signaling in the Collecting Duct

Fei Wang, Xiaohan Lu, Chuanming Xu, Kexin Peng, Tianxin Yang, Univ of utah, salt lake city, UT

We have shown that activation of (pro)renin receptor (PRR) and local renin response in the collecting duct (CD) contributes to AngII-induced hypertension. Moreover, the 28 kDa soluble (pro)renin receptor (sPRR) derived from cleavage of the extracellular domain of PRR is elevated by AngII and stimulated AQP2 expression via activation of frizzled-8/β-catenin pathway. Here we examined sPRR regulation of
ENaC and further explored its implication in AngII signaling. In cultured mpkCCD cells, a recombinant histidine-tagged rat sPRR, sPRR-His, at 10 nM for 24 h induced a 3-fold increase in α-ENaC protein abundance. The native sPRR generated by immunoprecipitation with anti-sPRR antibody from either mouse or human urine exhibited a similar stimulatory effect on α-ENaC expression. The sPRR-His-induced α-ENaC expression was nearly completely abolished by a frizzled-8 inhibitor OMP54F03 (OMP). Similarly, amiloride-sensitive Na+ transport as assessed by epithelial volt-ohmmeter was elevated by exposure to sPRR-His within minutes, which was abolished by OMP. In mpkCCD cells expressing a β-catenin-driven luciferase construct, the reporter activity was increased by 2.5-fold which was sensitive to OMP. In these cells, ENaC activity was transiently stimulated by exposure to 100 nM AngII within minutes, which was abolished by a sPRR neutralizing antibody. ELISA demonstrated that 24-h AngII treatment induced a 7-fold increase in medium sPRR concentrations. In floxed mice, urinary sPRR excretion was increased by AngII infusion whereas CD-specific PRR KO mice exhibited a reduced baseline level of urinary sPRR excretion which was not responsive to AngII infusion (300 ng/kg/min). Radiotelemetry demonstrated that the null mice were largely resistant to AngII-induced hypertension (MAP: Floxed/CTR: 105.9±1.6; Floxed/AngII: 136.3±3.1; KO/CTR:106.3±3.6; KO/AngII: 118.7±3.1 mmHg) which was fully restored after sPRR-His infusion via catheterization of jugular vein at 30 µg/kg/d (MAP: KO/AngII+sPRR-His: 135±7.5 mmHg). The MAP returned to normal when sPRR-His was terminated. Together, the present study reveals frizzled-8/β-catenin-dependent activation of α-ENaC in response to sPRR that at least in part contributes to AngII-induced hypertension.


Funding: No
Funding Component: 125

**Interleukin 6 Activates the Mineralocorticoid Receptor via Rac1, Increasing Sodium Chloride Cotransporter Expression and Activity**

Brandi M Wynne, Emory Univ, Atlanta, GA; Henrieke J van Elst, Radboud Univ, Nijmegen, Netherlands; Trinity A Kronk, Gillian Hecht, Otor Al-Khalili, Emory Univ, Atlanta, GA; Benjamin Ko, Univ of Chicago, Chicago, IL; Douglas C Eaton, Robert S Hoover, Emory Univ, Atlanta, GA

Hypertension is characterized by increased sodium (Na+) reabsorption along the aldosterone-sensitive distal nephron (ASDN), as well as a chronic systemic inflammation. Interleukin-6 (IL-6) is thought to be a mediator of this inflammatory process. Interestingly, increased Na+ reabsorption within the ASDN does not always correlate with increases in serum aldosterone (Aldo), the hormone that modulates Na+ reabsorption via the mineralocorticoid receptor (MR). Thus, understanding how increased MR-mediated Na+ reabsorption may occur independent of Aldo stimulation is critical. We hypothesize that IL-6 transactivates the MR via Rac1, increasing sodium chloride cotransporter (NCC) expression and activity. To demonstrate IL-6-mediated MR activation via Rac1, increasing sodium chloride cotransporter (NCC) expression and activity. To demonstrate IL-6-mediated MR activation via Rac1, mDCT15 cells were transfected with mineralocorticoid response element (MRE)-luciferase reporter constructs, treated with vehicle, Aldo (100nM), IL-6 (100ng/mL) and/or Rac1 inhibitor (EHT1864, 20uMol), and luciferase assays performed (48hrs). To determine if IL-6 increases NCC expression, in vivo, we perfused kidneys with IL-6 (intra-renal, 16ng/hr, 1-3d) and the cortex.
was isolated for WB analysis of total NCC expression. To determine direct IL-6 mediated effects on NCC activity, we performed thiazide-sensitive $^{22}$Na$^+$-uptake studies in cell monolayers in presence of: vehicle, IL-6 (100ng/mL for 45min or 6hrs) or IL-6+MR antagonist (spironolactone, 20uMol). IL-6 treatment significantly increased luciferase activity (2.6±0.9 fold/MRE-only), which was reduced with Rac1 inhibition (0.2±0.08, p<0.01, n=8-10) demonstrating a Rac1-dependent activation of MR via IL-6. In the kidney cortex, IL-6 infusion increased total NCC expression (>2.5 fold) after day 3, as compared to vehicle. In addition, $^{22}$Na$^+$-uptake studies (n=6) revealed an IL-6-mediated increase in Na$^+$ transport (1927±40 nmol/mg/20min vs. 1503±7 nmol/mg/20min, p<0.0001), which was reduced with the MR antagonist (1721±25nmol/mg, p<0.001). These data suggest that IL-6 activates the MR via Rac1, leading to increased NCC expression and activity. These data provide evidence for alternate mechanisms of increased ASDN Na$^+$ uptake during inflammatory and hypertensive conditions, independently of Aldo-mediated signaling.


Funding: No

Funding Component: 126

The Novel Quick, Accurate and Sensitive Measurement of Plasma Aldosterone and Active Renin Concentrations Will Be Beneficial for Diagnosing Primary Aldosteronism and for Drug Selection

Fumitoshi Satoh, Div of Clinical Hypertension, Endocrinology & Metabolism, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Ryo Morimoto, Div of Nepgrology, Endocrinology and Vascular Med, Tohoku Univ Hosp., Sendai, Japan; Yoshikiyo Ono, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Hosp, Sendai, Japan; Yoshitsugu Iwakura, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Hosp, Sendai, Japan; Masahiro Nezu, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Hosp, Sendai, Japan; Kei Omata, Yuta Tezuka, Div of Clinical Hypertension, Endocrinology & Metabolism, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Yasuhiro Igarashi, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Hosp, Sendai, Japan; Masataka Kudo, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Hosp, Sendai, Japan; Celso E Gomez-Sanchez, Div of Endocrinology, Univ of Mississippi Medical Ctr, Jackson, MS; Sadayoshi Ito, Div of Nephrology, Endocrinology and Vascular Med, Tohoku Univ Hosp, Sendai, Japan

The measurement of plasma aldosterone concentration (PAC) and renin activity (PRA) or active renin concentration (ARC) is clinically important not only for detection of primary aldosteronism (PA) but also for the selection of antihypertensive agents to treat patients successfully. However, it has taken about 7 days for clinicians to get the results. Of late, we developed the novel rapid assays of PAC and ARC, which are measurable in 10 minutes. We intended to investigate the utility and accuracy of the new methods. Both PAC and ARC were simultaneously measured by chemiluminescent enzyme immunoassay (CLEIA) system machine with their specific monoclonal antibodies and were automatically washed by the immobilized magnetic particles. We compared RIA-assayed PAC, PRA, ARC and LC-MS/MS-measured PAC with CLEIA-measured PAC and ARC in patients with PA (n=125) and essential hypertension (n=75). Measurements of PAC by CLEIA were significantly correlated with those of LC-MS/MS (Spearman's r = 0.988, p< 0.0001).
Measurements of PAC by RIA were also correlated with those of LC-MS/MS (Spearman's $r = 0.963$, $p < 0.0001$) and the degree of correlation was better in the comparison between CLEIA and LC-MS/MS. Bland-Altman plot analysis revealed the bias of 13.7 and the limits of agreement were 10.85 and 16.55 with 95% confidence interval when comparing CLEIA and LC-MS/MS. The comparison between RIA and LC-MS/MS revealed the bias of 33.4 with the limits of agreement of 15.2 and 51.5. There was the smaller systemic error in CLEIA when compared to RIA. Measurements of ARC by CLEIA were significantly correlated with those by RIA (Spearman’s $r = 0.930$, $Y = 0.960 X + 1.128$, $p < 0.0001$). The bias of -0.97 and the limits of agreement were -1.087 and -0.8671 with 95% confidence interval when comparing CLEIA-ARC and RIA-ARC. The lower detection limit of CLEIA-ARC was 0.25 pg/mL and much lower than that of RIA-ARC (2 pg/mL). CLEIA-ARC were also correlated with those of RIA-PRA (Spearman’s $r = 0.912$, $Y = 4.082 X + 0.549$, $p < 0.0001$). The bias of -0.97 and the limits of agreement were -1.087 and -0.8671 with 95% confidence interval when comparing CLEIA-ARC and PRA. The 10 minutes CLEIA measurements of PAC and ARC will be beneficial for diagnosing PA or for medication, because of their quickness, accuracy and sensitivity.

Enhanced activation of mineralocorticoid receptors (MRs) impairs insulin metabolic signaling, increases oxidative stress, and induces inflammation with associated cardiovascular abnormalities. Our previous data in female mice suggests that activation of endothelial cell MRs (ECMR) contributes to development of vascular stiffness partly by impairing insulin metabolic signaling and reducing endothelial derived nitric oxide (NO) production. Emerging information suggests that microRNA 103 (miR103) is upregulated and thus promotes endothelial inflammation and atherosclerosis in both ob/ob mice and western diet-induced obese C57BL/6J mice. However, the interaction of ECMR and miR103 in the promotion of vascular inflammation and stiffness has not been explored. We hypothesized that ECMR signaling prompts vascular inflammation and stiffness through miRNA-103-mediated suppression of Kruppel-like factor-4 (KLF4). Female ECMR knockout (ECMR$^{-/-}$) and wild-type littermate females were treated with aldosterone (Aldo) (250 µg/kg/day) via osmotic minipumps for 4 weeks. Aldo infusion induced endothelium stiffness and impaired aortic relaxation in wild-type mice as determined by ex vivo atomic force microscopy and wire myograph techniques, respectively. The elevated aortic stiffness and impaired relaxation was accompanied by increases in expression of miR103 and intercellular adhesion molecule 1 (ICAM-1) and a reduction in phosphorylation of serine (Ser) 1177/activation of endothelial NO synthase (eNOS). These Aldo-induced endothelial abnormalities were
prevented in ECMR⁻/⁻ mice. Furthermore, application of a miR103 inhibitor to ECs in vitro attenuated Aldo (10⁻⁸ M)-induced a decrease in KLF4 expression, which has anti-inflammatory functions mediated by upregulation of phosphorylation of eNOS and downregulation of ICAM-1. These findings suggest that increased ECMR signaling and associated miR103 activation plays a key role in Aldo-induced KLF4 suppression and associated vascular inflammation and aortic stiffness in females.

G. Jia: None. J. Habibi: None. A. Aroor: None. Z. Sun: None. V. DeMarco: None. G. Meininger: None. I. Jaffe: None. J. Sowers: None.

Funding: No

Funding Component: 128

**Leptin: A Regulator of Aldosterone Synthase Expression & Aldosterone Secretion in Visceral Adipocytes, in Mice**

Eric J Belin de Chantemele, Anne-Cecile Huby, P. Thomas Menk, Weiqin Chen, Brian Lane, Galina Antonova, Medical Coll of Georgia at Augusta Univ, Augusta, GA

Obesity is associated with inappropriately high aldosterone levels, which contribute to the development of metabolic and cardiovascular disorders. The origin of these high aldosterone levels is incompletely understood. We recently demonstrated that the adipocyte-derived hormone leptin regulates aldosterone synthase (CYP11B2) expression and stimulates aldosterone release from adrenal zona glomerulosa cells. Recent studies demonstrate that adipocytes express CYP11B2 and secrete aldosterone. However, the mechanisms regulating aldosterone release from adipocytes remain unclear. Likewise, whether visceral (Visc) and subcutaneous (SubQ) adipose tissue contribute to a similar extent to aldosterone production is unknown. We tested the hypothesis that leptin increases adipocyte CYP11B2 expression and aldosterone production and investigated whether Visc and SubQ adipose tissues respond similarly to leptin. Immunostaining of mouse adipose tissue cross-sections and isolated mature adipocytes revealed that Visc and SubQ adipose tissue express leptin receptors. Treatment of mouse freshly isolated mature adipocytes, non-differentiated (stromal fraction) and differentiated adipocytes revealed that leptin dose-dependently increased CYP11B2 expression and aldosterone production in Visc adipose tissue only. Although leptin receptor and CYP11B2 levels were similar in SubQ and Visc adipocytes, SubQ adipocytes were unresponsive to leptin. The physiological relevance of these in vitro data was tested by measuring plasma aldosterone levels in mice deprived of adipose tissue (lipodystrophic mice) treated with leptin. Absence of adipose tissue in lipodystrophic mice blunted leptin-induced increases in aldosterone levels (WT-vehicle: 471±82 vs. WT-Leptin: 1699±396, p<0.05; KO-vehicle: 539±71 vs. KO+leptin: 787±156, NS).

The human relevance of these data was determined by reporting that CYP11B2 expression gradually increased with body mass index in human mediastinal and omental fat depots. In summary these data strongly suggest that leptin regulates CYP11B2 levels and aldosterone release in visceral adipose tissue and that leptin-induced, adipocyte-derived aldosterone may contribute to obesity-associated hyperaldosteronism.


Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)
The Role of Intron-2 Conversion Polymorphism in the Regulation of Human Aldosterone Synthase Gene Expression

Brahmaraju Mopidevi, Sravankumar Perla, Indu Sivankutty, Ashok Kumar, The Univ of Toledo, Toledo, OH

The hCYP11B2 gene encodes aldosterone synthase, the rate-limiting enzyme in the biosynthesis of aldosterone. Inappropriate excess levels of aldosterone induces positive sodium balance and predisposes to hypertension and other cardiovascular problems. hCYP11B2 has a T/C polymorphism located at -344 in its promoter region. This variant is in close linkage disequilibrium with a complex hCYP11B2 Intron 2 conversion polymorphism (IC), which replaces a segment of DNA within intron 2 with the corresponding region of hCYP11B1. Epidemiological studies have suggested that -344 T/C and the IC polymorphism associating with hypertension. The IC polymorphism always occur when the promoter contains -344T variant. In the present study we have for the first time sequenced the full length hCYP11B2 intron 2 from randomly selected hypertensive samples from the Caucasian cohort and identified the segment of hCYP11B2 replaced by that of hCYP11B1 Intron2. It is interesting to note that in these genomic DNA samples there is no change in the hCYP11B1 intron2 sequence. In silico transcription factor binding site predictions indicate that there are several transcription factors which can bind more strongly to the hCYP11B2 IC region as compared to wild type intron2 (wt). We have cloned the IC polymorphism and the wt intron nucleotide sequences in front of the 1kb hCYP11B2 promoter containing -344T. These reporter constructs showed differential transcriptional activation. Basal promoter activity of IC intron was increased by 1.94 fold (p<0.05) compared to that of wt intron2. Further co-transfection studies of these reporter constructs along with transcription factors HNF3B and NFkB showed significantly increased relative luciferase activity of 2.9 and 3.26 fold (p<0.05) in IC construct as compared to wt intron respectively. These results strongly suggest that IC in hCYP11B2 has strong regulatory elements as compared to the wt intron leading to enhanced transcriptional activity and thus increased hCYP11B2 gene expression. This would increase tissue or plasma aldosterone levels in human subjects containing IC polymorphism. The long term consequence of altered regulation of aldosterone production can lead to an increase in blood pressure and cardiovascular complications.

B. Mopidevi: None. S. Perla: None. I. Sivankutty: None. A. Kumar: None.

Funding: No

Funding Component: 130

Modulation of Salt and Mineralocorticoid Sensitivity of Blood Pressure by the Circadian Clock Protein Per1

Kristen Solocinski, Xuerong Wen, Kit-Yan Cheng, Jeanette Lynch, Brian D Cain, Charles S Wingo, Michelle L Gumz, Univ of Florida, Gainesville, FL

The circadian clock is important for maintaining rhythms in physiological functions including blood pressure (BP). Circadian disruption leads to increased disease risk. The clock has also been implicated in the maintenance of a normal dip in BP at night. In humans, non-dipping (night/day difference in BP<10%) is associated with an increased risk of cardiovascular and kidney disease. Dipping status can also be affected by salt intake and by hormones such as the mineralocorticoid aldosterone. The goal of
this study was to determine the effects of a high salt (HS, 4% NaCl) diet plus mineralocorticoid (deoxycorticosterone pivalate (DOCP)) on BP regulation by the circadian clock protein Per1 in C57BL/6J mice. BP was monitored in conscious, unrestrained male mice by radiotelemetry and values are reported as mean arterial pressure (MAP) ± SEM. Under control conditions, MAP in male WT mice was 112.5 ± 1.08 mmHg during the night when mice are active and decreased to 102.1 ± 1.7 mmHg during the day, a “dip” in MAP of 9.2 ± 1.3%. Similarly, Per1 KO mice dip 14 ± 1.4%, with night time MAP of 119.8 ± .9 mmHg which decreased to 103 ± 1.4 mmHg during the day. On HS/DOCP, WT mice MAP decreased from 114.5 ± 1.1 mmHg to 101.5 ± 1.92 mmHg (night indicated by shaded bars in figure). This 11.4 ± 1.9% dip in WT mice was not significantly different from what was observed under control conditions. In contrast, Per1 KO mice display a significantly attenuated dip of 5.7 ± 1.4% with night time MAP of 125.3 ± 1.5 mmHg dropping to 118.1 ± 1 mmHg during the inactive day period (p<0.05). Thus, HS/DOCP treatment in Per1 KO mice leads to non-dipping hypertension. This is the first report of this phenotype in a single clock gene KO.

K. Solocinski: None. X. Wen: None. K. Cheng: None. J. Lynch: None. B.D. Cain: None. C.S. Wingo: None. M.L. Gumz: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH DK085193, DK098460.

Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

Quantification of in vivo Placental Oxygenation Using Ultrasound-guided Spectral Photoacoustic Imaging

Carolyn Bayer, Tulane Univ, New Orleans, LA; Geoffrey P Luke, Dartmouth Univ, Hanover, NH; Jason R Cook, The Univ of Texas at Austin, Austin, TX; Stanislav Y Emelianov, Georgia Inst of Technology, Atlanta, GA

Preeclampsia, linked to abnormal placental development and ischemia, is a major cause of fetal and maternal death. Currently, the only cure is high-risk preterm delivery. Although clinical therapies to regulate the symptoms of preeclampsia—mean arterial pressure and proteinuria—it is unknown whether these therapeutics restore placental blood flow and reduce ischemia. We are developing ultrasound-guided spectral photoacoustic imaging to quantify placental ischemia and characterize therapeutic response. Ultrasound imaging is the preferred imaging modality to monitor pregnancy due to its safety, low cost, and mobility. Similar to ultrasound, photoacoustic imaging can provide co-registered images of tissue function. In these studies, pregnant SWV mice were imaged longitudinally from E12.5 to 18.5 using a Vevo LAZR small animal imaging system. Algorithms were developed to correlate the spectral photoacoustic data to a hemoglobin oxygenation (%sO2) calibration standard—a phantom containing blood at varying partial pressures of oxygen. The phantom calibration standard deviation was 7.9% (n=3). The
resulting ultrasound images were used to segment the placenta (Figure 1). An overlay of the %sO₂ on the ultrasound image maps placental variation in %sO₂ during longitudinal development. We demonstrate that our imaging method is capable of quantifying %sO₂ and monitoring placental function in vivo. Future work will use our preclinical %sO₂ quantification methods to characterize placental function during treatment for preeclampsia.


Funding: No

Funding Component: 132

Adrenergic Receptor Blockade Prevents Placental Ischemia-induced Hypertension

Frank T Spradley, Joey P Granger, Univ of Mississippi Medical Ctr, Jackson, MS

Hypertensive disorders of pregnancy are the number one cause of pregnancy-related deaths in the United States. Preeclampsia is a disorder of maternal hypertension and cardiovascular dysfunction typically presenting in the second half of pregnancy along with fetal growth restriction. There are no steadfast therapies besides early delivery of the fetus and ischemic placenta, which releases factors into the maternal circulation promoting hypertension. Although sympathetic nervous activity was found to be increased in preeclamptic versus normal pregnant women, it is unknown if sympathetic nervous system plays a role in placental ischemia-induced hypertension. To address this question, we tested the hypothesis that adrenergic receptor blockade prevents placental ischemia-induced hypertension.

Wistar rats were randomized to receive reduced uterine perfusion pressure, (RUPP, n=6) or Sham (n=5) surgeries on gestational day 14 and examined at day 19. In RUPP vs Sham rats, respectively, mean arterial blood pressure (115 ± 4 vs 103 ±2 mmHg, P<0.05) and the number of absorbed fetuses (6 ± 1 vs 1 ± 1, P<0.05) were greater whereas average fetal weight was lower (1.7 ± 0.1 vs 2.0 ± 0.2, P<0.05) with similar placental weights (0.49 ± 0.03 vs 0.52 ± 0.03). In RUPP vs Sham rats, renal cortical norepinephrine content (HPLC) was higher (183 ± 15 vs 150 ± 8 pg/mg wet weight, P<0.05) and vasoconstriction to phenylephrine was greater in small, third order mesenteric arteries (Emax: 262 ± 19 vs 160 ± 26% of KCl response). A subset of RUPP rats (n=3) received terazosin and propranolol (3 mg/kg per day each, subcutaneous osmotic minipump) to block alpha- and beta-adrenergic receptors, respectively, beginning the day of RUPP surgery. At day 19, adrenergic blockade prevented the development of hypertension (100 ± 4 mmHg, P<0.05) and did not alter number of fetal absorptions (8 ± 1). Average fetal weight was higher (2 ± 0.1, P<0.05) and placental weight lower (0.41 ± 0.03, P<0.05) compared to the untreated RUPP rats. In conclusion, placental ischemia-induced hypertension depends on activation of the sympathetic nervous system. The mechanism for this enhanced sympathetic nerve activity is unknown but may involve factors released from the ischemic placenta.

F.T. Spradley: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; P20GM104357, 2 P01 HL051971. J.P. Granger: B. Research Grant
Depletion of Natural Killer Cell Activation in Response to Placental Ischemia Improves Hypertension and Intrauterine Growth Restriction During Pregnancy

Denise C Cornelius, Jamil Elfarra, Lorena M Amaral, Maggie McCalmon, Mark W Cunningham Jr., Tarek Ibrahim, Jeremy D Scott, Babbette LaMarca, Univ of Mississippi Medical Ctr, Jackson, MS

Women with preeclampsia (PE), newly developed hypertension and renal dysfunction during pregnancy, have small-for-gestational-age babies and demonstrate an increase in the cytolytic natural killer (NK) cell activation. The specific role of cytolytic NK cells in the pathophysiology of PE has not been clearly defined. The reduced uterine perfusion pressure (RUPP) model of placental ischemia (PI) exhibits many of the characteristics of PE including hypertension, renal dysfunction, chronic inflammation and intrauterine growth restriction (IUGR). In this study, we tested the hypothesis that PI results in cytolytic activation of NK cells, and examined a role for a reduction in NK cells in RUPP to attenuate PE-like characteristics in response to PI. In this study NK cells were depleted in RUPP rats by intraperitoneal administration of the Anti-asialo GM1 antibody on gestation days 15 and 17. PBMCs and placental lymphocytes were examined via flow cytometry to quantify cytolytic NK cells and to verify NK cell depletion, blood pressure (MAP) and pup weight were measured. While total placental NK cells numbers did not change in response to PI (NP: 14±4.5%; RUPP: 14.3±3.8%), cytolytic activation of placental NK cells significantly increased in response to PI (NP: 3.4±1.1% vs RUPP 10.0±3.4%; p<0.05). Moreover, depletion of NK cells in RUPP (total NK: RUPP: 14.3±3.8% vs RUPP+NKD: 3.5±0.9%) significantly improved blood pressure and intrauterine growth restriction (IUGR): MAP significantly increased in response to PI from 109.5±2.3 mmHg in NP (n=10) to 125.4±2.7 mmHg (n=9) in RUPP rats (p<0.001). Depletion of NK cells with the cell specific depleting antibody significantly lowered blood pressure to 114.4±1.9 mmHg in RUPP+NKD rats (n=11, p<0.01). Additionally, NK cell depletion in RUPP significantly reduced IUGR in response to PI (1.8±0.4g in RUPP+NKD vs 2.0±0.4g in RUPP+NKD; p<0.05). Depletion of NK cells in RUPP rats was positively associated with lowering blood pressure and blunting IUGR in response to PI. These results suggest a role for cytolytic NK in contributing to hypertension and IUGR in response to PI, potentially identifying previously unknown mechanisms of PE pathophysiology and new therapeutic targets to improve maternal and fetal outcomes of PE.

D.C. Cornelius: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; American Heart Association 16SDG27520000. J. Elfarra: None. L.M. Amaral: None. M. McCalmon: None. M.W. Cunningham: None. T. Ibrahim: None. J.D. Scott: None. B. LaMarca: None.
Katryn Paquette, Thuy Mai LUU, Anik Cloutier, Marie-Amélie Lukaszewski, Mariane Bertagnolli, Dept of Pediatrics, Sainte-Justine Univ Hosp and Res Ctr, Univ of Montreal, Montreal, QC, Canada; Ramy El-Jalbout, Dept of Medical Imaging, Sainte-Justine Univ Hosp and Res Ctr, Univ of Montreal, Montreal, QC, Canada; Anne-Laure Lapeyraque, Anne-Monique Nuyt, Dept of Pediatrics, Sainte-Justine Univ Hosp and Res Ctr, Univ of Montreal, Montreal, QC, Canada

Background: Children born extremely preterm (EPT; ≤29 weeks) have higher blood pressure (BP), lower nephron mass, and increased risk in later life of renal and cardiovascular dysfunction. Whether nephron mass and renal function impact BP in EPT subjects is unknown. We correlated BP with renal size and function in young adults born EPT vs term (T).

Methods: Anthropometric measurements, serum and urine chemistry, renal ultrasound, and 24 hour ambulatory BP were obtained in 40 EPT and 40 T born young adults matched for age, sex, race, and socioeconomic status. Comparisons were made using paired T and Wilcoxon signed ranked tests and correlations using Pearson correlation.

Results: Study population characteristics are in Table 1. Young adults born EPT had higher systolic BP (SBP) and diastolic BP (DBP), and smaller kidneys (Table 2). Awake SBP and DBP loads in the hypertensive range inversely correlated with kidney size only in EPT participants.

Table 1 – Study population characteristics

<table>
<thead>
<tr>
<th></th>
<th>EPT</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age ±SD, weeks</td>
<td>27.4±1.2</td>
<td>39.6±1.1</td>
</tr>
<tr>
<td>Birth weight ±SD, g</td>
<td>1005±123</td>
<td>3120±31</td>
</tr>
<tr>
<td>Neonatal length of hospital stay ±SD, days</td>
<td>78±28</td>
<td>3±7±1.2</td>
</tr>
<tr>
<td>Oxygen use at 36 weeks</td>
<td>35.1%</td>
<td>-</td>
</tr>
<tr>
<td>Intraventricular hemorrhage</td>
<td>19.4%</td>
<td>-</td>
</tr>
<tr>
<td>Age at participation ±SD, years</td>
<td>23.6±2.2</td>
<td>23.3±2.0</td>
</tr>
<tr>
<td>Body mass index ±SD, kg/m²</td>
<td>22.1±3.0</td>
<td>23.1±4.2</td>
</tr>
</tbody>
</table>

Conclusion: Young adults born EPT have higher BP and smaller kidneys vs T controls. EPT young adults with smaller kidneys have a greater BP load in the hypertensive range.


Funding: No

Funding Component: 135

Obesity Accelerates the Deterioration of Renal Function in Developmental Programming of Hypertension. Role of Angiotensin II and Oxidative Stress

Antonio Tapia, Juan M Moreno, Maria T Llinas, F. Javier Salazar Sr., Univ of Murcia, Murcia, Spain

Numerous studies have shown gender-dependent differences in the deterioration of renal function in models of developmental programming of hypertension (DPH). It is also known that obesity is associated to changes in renal function and that both angiotensin II (Ang II) and oxidative stress are involved in the renal alterations that occur in obesity and in animals with DPH. The main objectives were to examine whether the increment of arterial pressure (AP) and the deterioration of renal function are accelerated as a consequence of obesity in SD
rats with DPH; whether these changes are gender-dependent; and to evaluate the role of Ang II and oxidative stress in these AP and renal function changes. A high fat diet (60%) was given during the first 4 months of age and DPH was induced by an AT receptor antagonist during nephrogenic period (ARAnp). Systolic AP (mmHg) was greater (P<0.05) in ARAnp-obese rats (167 ± 3 in ♂; 146 ± 4 in ♀) than in ARAnp (155 ± 3 in ♂; 137 ± 3 in ♀); obese (147 ± 2 in ♂; 137 ± 2 in ♀) or control (127 ± 1 in ♂; 119 ± 2 in ♀) rats. Three days administration of candesartan (7 mg/kg/day) led to a decrease in AP that was greater (P<0.05) in ARAnp-obese rats (55 ± 3 in ♂; 45 ± 4 in ♀) than in ARAnp (40 ± 3 in ♂; 37 ± 4 in ♀); obese (38 ± 4 in ♂; 27 ± 4 in ♀) or control (12 ± 2 in ♂; 14 ± 3 in ♀) rats. The acute Ang II infusion (30 ng/kg/min) induced an increase in renal vascular resistance (mmHg/ml/min/gr kw) that was also greater in ARAnp-obese rats (217 ± 45% in ♂; 145 ± 38% in ♀) than in ARAnp (103 ± 9% in ♂; 97 ± 8% in ♀); obese (106 ± 14% in ♂; 106 ± 17 in ♀) or control (51 ± 7% in ♂; 51 ± 10% in ♀) rats. The response to candesartan or Ang II infusion in ARAnp-obese rats was gender-dependent and may be explained by an enhanced oxidative stress. The expression of P67phox in the renal cortex was greater (P<0.05) in ARAnp-obese rats (3,00 ± 0,05 in ♂; 2,60 ± 0,04 in ♀) than in ARAnp (1,16 ± 0,04 in ♂; 1,66 ± 0,03 in ♀); obese (0,94 ± 0,06 in ♂; 1,02 ± 0,02 in ♀) or control (1,00 ± 0,02 in ♂; 1,02 ± 0,023 in ♀) rats. The results of this study suggest that obesity at an early age enhances the hypertension and accelerates the deterioration of renal function that occurs when cardiovascular disease is programmed during the perinatal period. It is also shown that Ang II and oxidative stress seems to play an important role in these AP and renal function changes.

A. Tapia: None. J.M. Moreno: None. M.T. Llinas: None. F. Salazar: None.

Funding: No

Influence of Race and Obesity on the Renin-Angiotensin-Aldosterone System in Adolescents Born Preterm


Background: While neonatal morbidity and mortality in preterm infants have improved dramatically, the long-term cardiovascular and renal consequences of prematurity are incompletely understood. Prematurity may induce programmed changes in the renin-angiotensin (Ang)-aldosterone system (RAAS), a key regulator of cardiovascular and renal function. Race and obesity influence the RAAS and may modify the effects of prematurity on the RAAS. We hypothesized that the RAAS differs by race and obesity in adolescents born prematurely with very low birth weight (VLBW).

Methods: A cohort of 173 adolescents with VLBW was evaluated at age 14. We measured renin, aldosterone, Ang II, and Ang-(1-7) in the plasma; Ang II, Ang-(1-7), and creatinine in the urine; and we calculated the aldosterone/renin ratio and the Ang II/Ang-(1-7) ratios. We used general linear regression models to estimate the difference in the RAAS according to overweight/obesity (body mass index ≥85% for age and sex) and race, adjusting for confounding variables. Results: On unadjusted analyses as well as analyses adjusted for sex, antenatal corticosteroid exposure, and
maternal hypertension, black race was associated with decreased urinary Ang-(1-7)/creatinine (adjusted estimate -0.18, 95% CI -0.36 to -0.01, p = 0.04) and decreased renin (-0.36, -0.68 to -0.05, p = 0.03). In analyses stratified by sex, black males, but not black females, had decreased renin (-0.63, -1.1 to -0.16, p = 0.01) and aldosterone (-0.61, -1.19 to -0.04, p = 0.04). Obesity was associated with increased urinary Ang II/(1-7) (0.29, 0.04 to 0.53, p = 0.02), decreased plasma Ang-(1-7) (-0.4, -0.8 to -0.002, p = 0.05), increased plasma Ang II (0.21, 0.03 to 0.39, p = 0.02), and increased plasma Ang II/(1-7) (0.61, 0.2 to 1.01, p = 0.004). Conclusions: In adolescents born with VLBW, there is racial variation in the RAAS. Black adolescents, especially males, have an altered renal RAAS and lower renin and aldosterone. Obesity is associated with a potentially deleterious alteration in the RAAS, with a shift in the renal and systemic RAAS towards Ang II and away from Ang-(1-7). These shifts in the RAAS associated with race and obesity may increase the risk of renal and cardiovascular disease in adolescents born with VLBW.

**A.M. South:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH Program Project Grant P01 HD047584. E. Honoraria; Modest; Alexion Pharmaceuticals. G. Consultant/Advisory Board; Modest; Alexion Pharmaceuticals. P.A. Nixon: None. M.C. Chappell: None. D.I. Diz: None. G.B. Russell: None. B.M. Snively: None. H.A. Shaltout: None. J.C. Rose: None. T.M. O'Shea: None. L.K. Washburn: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH Program Project Grant P01 HD047584.

**Interaction of the Mineralocorticoid Receptor with RACK1**

**Maniselvan Kuppusamy,** Elise P Gomez-Sanchez, Celso E Gomez-Sanchez, Univ of Mississippi Medical Ctr, Jackson, MS

The mineralocorticoid receptor (MR) is a multifunctional ligand dependent transcription factor in the steroid receptor superfamily. The MR mediates aldosterone regulation of electrolytes and blood pressure. MR transcriptional co-regulators to influence specific gene transcription. We used a yeast two-hybrid system to find proteins that interact with a full-length MR as bait. Results: Among other proteins, we found a specific interaction of MR with RACK1 (Receptor for Activated C Kinase 1), a scaffolding protein. Overexpression of RACK1 using a tetracycline-inducible lentivirus in mouse cortical collecting duct M1 cells stably expressing a reporter Gaussia luciferase gene under a hormone-response element promoter resulted in enhanced agonist-dependent MR transactivation. Inhibition of RACK1 protein expression by short hairpin RNA led to a significant reduction in MR activation of the reporter gene. RACK1 regulation of MR action is mediated through the PKC-β signaling pathway. MR and RACK1 co-precipitated using a MR antibody in Sprague-Dawley brain tissue and M1 cells and immunofluorescent histochemistry showed MR and RACK1 co-localization in Male Sprague-Dawley brains and M1 cells. Conclusion: The scaffolding protein RACK1 is associated with MR under basal and agonist stimulated conditions and facilitates agonist stimulated MR actions through PKC-β. These findings indicate that RACK1 function as a novel co-activator of MR.

**Funding Component:** P100

**Funding:** No
Environmental influences acting early in life predispose to premature cardiovascular disease. In line with this concept, assisted reproductive technologies (ART) cause premature vascular ageing and arterial hypertension in mice and humans, but the underlying mechanisms are incompletely understood. In rodents, pathological events during pregnancy cause arterial hypertension in the offspring by increasing the vascular responsiveness to angiotensin II (ANG II). We speculated that a similar mechanism could be involved in ART-induced arterial hypertension. In aortic ring preparations of ART and control mice, we, therefore, assessed the vasoconstrictor responsiveness to stepwise increasing doses of ANG II in the presence of the eNOS inhibitor L-NMA. We also examined ANG II receptor (AGTR) type 1 and 2 expression (Western Blot) and AGTR gene promoter methylation (bisulfite sequencing) in the aorta. Finally, we measured mesenteric-artery responsiveness to acetylcholine and arterial blood pressure (carotid catheter). As expected, ART mice displayed endothelial dysfunction (P=.03, vs. control) and arterial hypertension (121.8±7.3 vs. 114.6±4.5 [mmHg], P=.02, vs. control). Most importantly, the vasoconstrictor response to ANG II, independently of endothelial function, was markedly increased in ART compared to control mice (0.32±0.05 vs. 0.22±0.04 [% of maximal KCl contraction], P<.01, vs. control). Moreover, and in line with this finding, in ART mice AGTR 1/AGTR 2 ratio of protein expression in the aorta was significantly increased (1.49±0.30 vs. 0.36±0.16, P<.008, vs. control) and the AGTR 1b gene promoter was hypomethylated compared with control mice (8.1±4.3 vs. 10.6±1.6 [% of methylation], P<.05, vs. control). Here, we show for the first time that ART increases the vasoconstrictor sensitivity to ANG II in the aorta. This is related to an epigenetically mediated imbalance between the expression of the vasoconstrictor (AGTR 1) and vasodilator (AGTR 2) ANG II receptor. Hence, we identified a new mechanism contributing to ART-induced premature vascular ageing and arterial hypertension in mice. We speculate that this
mechanism also contributes to ART-induced premature vascular ageing and arterial hypertension in humans.


Funding: No
Funding Component: P102

**Differences in Angiotensin-(1-7)/alamandine-mediated Signaling in Tumoral and Normal Cell Lines**

Walkyria O Sampaio, Marilene Luzia Oliveira, Felipe A Silva, Leticia B Silva, Gisele M. Etelvino, Danielle G Passos-Silva, Robson A Santos, Federal Univ of Minas Gerais, Belo Horizonte, Brazil

Previously studies have demonstrated that besides its actions in the cardiovascular system, Angiotensin-(1-7) also plays a role in inhibiting tumoral growth. The role of recently described Alamandine in this field is not clear. The signaling pathways underling anti-tumoral actions of these peptides are also poorly understood. Therefore, the aim of this study was to elucidate the modulatory effect of Ang-(1-7) and Alamandine in the PI3K cascade, a well-known signaling pathway described to be involved in proliferation and cancer. To achieve this goal, we stimulate human pancreatic and lung cancer cell lineage (Miapaca and A549), as well as a control cell lineage (VERO) with Ang-(1-7) and Alamandine. Through western blotting analysis, our data suggest that both Ang-(1-7) and Alamandine activate the phosphatase pTEN (dephosphorylation of S380/T382/T383) (48±4% after 24 hours Ang-(1-7) treatment in miapaca and A549 in comparison of non-treated cells, p<0.05), which dephosphorylates PI3K, inactivating this kinase. Furthermore, AKT phosphorylation is transient, followed by a significant dephosphorylation when compared to the non-treated cells (30±5% after 24 hours Ang-(1-7) treatment in miapaca in comparison of non-treated cells, p<0.05). Ang-(1-7) also inhibits a PTEN downstream effector kinase, mTOR through dephosphorylation of T246 (70±5% after 24 hours Ang-(1-7) treatment in miapaca in comparison of non-treated cells, p<0.05). These effects were not observed in control non-tumoral cells (VERO cells). As previously demonstrated with Ang-(1-7) stimulation, Alamandine also induces the FOXO1 activation and migration to the nucleus in A549 (122 ± 8 of A.U. of fluorescence at 4 hours after alamandine treatment vs 46±4 A.U. at control, p<0.001) and Miapaca cells (67 ± 5 of A.U. of fluorescence at 4 hours after alamandine treatment vs 16±2 A.U. at control, p<0.001). These results indicate that, in contrast to normal tissues, Ang-(1-7) and Alamandine decreases, through PTEN activation, PI3K/AKT pathway in tumoral cells.


Funding: No
Funding Component: P104

**Role of Adenosine Receptors in Angiotensin II Dependent Hypertension in Mice**

Vishal R Yadav, Zhichao Zhou, Bunyen Teng, S. Jamal Mustafa, West Virginia Univ, Morgantown, WV

Our recent finding showed that acute angiotensin (ANG) II stimulation enhanced A1
adenosine receptor (AR) dependent
cytochrome P450 (CYP) 4A mediated
contraction in mouse mesenteric arteries (MA).
In addition, A2BAR dependent and partly KATP
channel mediated relaxation was also reduced
with ANGII stimulation. These data suggest a
possible interaction between ANG II and ARs at
vascular level which may play a role in ANG II
dependent hypertension.
We developed ANG II (1000 ng/kg/min, 21 days)
infused hypertension mouse model and tail cuff
was used to measure blood pressure. Aorta as a
conduit and MA (second order) as a resistance
artery were chosen for muscle tension studies.
Protein expressions in aortas and MAs were
analyzed by western blot.
Infusion of ANG II increased systolic arterial
blood pressure (SAP) in mice. Particularly, in
ANG II infused mice, SAP (in mmHg, mean ± SD)
at day 0 was 101.6 ± 6.675 which increased to
156.7 ± 15.68 on day 21 (p<0.05) while in saline
control mice, SAP was 100.8 ± 6.753 (day 0) and
changed to 105.4 ± 7.620 (day 21).
Higher expression of A1AR (~155% over 100%)
and CYP4A (~50%, ~0.6 over 0.3, ratio to actin)
was present in MAs in hypertensive compared
to control mice. Expression of A1AR and CYP4A
was comparable in aortas of hypertensive and
control mice.
CCPA (A1AR agonist) -induced concentration
dependent contraction (% normalize to
phenylephrine contraction) was significantly
reduced (~15% from 40% at 100 nM) in aorta of
hypertensive mice compared to control. NECA
(non-selective AR agonist) produced a
significantly higher (~ -15% from 0% at 10 µM)
relaxation in aorta of hypertensive mice. In
MAs, CCPA-induced contraction was reduced in
hypertensive mice. NECA induced relaxation
was comparable in MAs. Pinacidil (KATP channel
opener) induced relaxation was significantly
lower (~0% from -25% at 100 nM) in MAs of
hypertensive mice.
In conclusion, our data reveal important role for
A1AR dependent signaling in ANG II induced
hypertension. Higher A1AR and CYP4A may
reduce KATP channel dependent relaxation of
MAs aiding vascular contraction and blood
pressure. Further studies involving ARs
pharmacological inhibitors and genetic models
are required to fully understand the
relationship between ANG II and ARs in the
development of hypertension.
V.R. Yadav: None. Z. Zhou: None. B. Teng:
None. S. Mustafa: None.
Funding: No
Funding Component: P105

Greater 24 Hour Blood Pressure Variability is
Associated With Higher 24 Hour Systolic Blood
Pressure and Glucose Independent of Age and
Large Elastic Artery Stiffness in Normotensive
Adults
Lyndsey E DuBose, Seth W. Holwerda, Amy K.
Stroud, Nealy A. Wooldridge, Janie E. Myers,
Kaitlyn M. Dubishar, Gary L. Pierce, Univ of
Iowa, Iowa City, IA

Older age is associated with elevated large
elastic artery stiffness, a strong predictor of
cardiovascular (CVD) risk in middle-age/older
(MA/O) adults independent of blood pressure
(BP). Greater 24-hour systolic BP variability
(BPV) is also an independent risk factor for CVD
and is linked to large artery stiffness in MA/O
adults with hypertension and diabetes.
However, its relation to age-related arterial
stiffness in adults with low risk factor burden is
unclear. We hypothesized that higher systolic
BPV would be: 1) associated with advancing
age, and 2) related to elevated aortic and
carotid artery stiffness among healthy MA/O
adults. To determine this, 98 healthy adults
(ages 19-70 yrs) with measurements of systolic
BPV (standard deviation of 24 hr systolic BP) via
ambulatory BP monitoring, aortic stiffness
(carotid-femoral pulse wave velocity, cfPWV),
carotid artery stiffness (β-stiffness via carotid tonometry/B mode ultrasound) and circulating metabolic factors were included. In the entire cohort, greater systolic BPV was not associated with age, cfPWV, carotid β stiffness or circulating lipids/glucose (all P>0.05), but was correlated (age-adjusted) with 24 hr systolic BP (r= 0.41, P<0.001) and BMI (r= 0.21, P<0.05). In stepwise linear regression analyses that included age, sex, BMI, only 24 hr systolic BP was associated with systolic BPV (β= 0.14 ± 0.03, Model R²= 0.20, P< 0.001). Interestingly, there was no difference in 24 hr systolic BPV (11.4 ± 0.4 vs 11.4 ± 0.5 SD mmHg, P=0.99) in young (n=55; 29.0 ± 0.7 yrs) vs. MA/O (n= 43; 53.0 ± 1.2 yrs) adults despite higher cfPWV (594 ± 12 vs 913 ± 39 cm/sec, P<0.001), carotid β-stiffness (6.8 ± 0.6 vs 9.3 ±0.9 U, P=0.001) and 24 hr systolic BP (121 ± 1 vs 125 ± 2 mmHg, P<0.05). Systolic BPV was associated with BMI (r= 0.42, p< 0.01) and fasting blood glucose (r= 0.54, P= 0.001) in MA/O but not young adults.

Charles German, Universtiy of Alabama at Birmingham, Birmingham, AL

Background
High dietary sodium intake and aldosterone excess have been independently linked to increased cardiovascular morbidity and mortality. In addition, a large body of literature indicates that aldosterone excess contributes importantly to antihypertensive treatment resistance and is associated with higher 24-hour ambulatory blood pressure (BP) levels.

Objective
This study was designed to determine if high dietary sodium intake, and hyperaldosteronism combine to mediate the development of abnormal diurnal BP patterns including nocturnal hypertension and dipping BP patterns.

Methods
A single-center cohort of 326 African American (AA) and Caucasian resistant hypertensive patients were prospectively evaluated by assessing 24-hr urinary aldosterone (UAldo), plasma renin activity (PRA), sodium (UNa+) levels, and 24-hr ambulatory blood pressure monitoring (ABPM). Daytime, night-time, and 24-hr BP and dipping patterns were determined. High sodium excretion was defined as UNa+ ≥ 200 mEq/24hr and hyperaldosteronism was defined as UAldo ≥ 12 μg/24hr and PRA ≤ 1 ng/ml/hr.

Results
There was no difference in ABPM and dipping patterns when comparing the normal versus high sodium group. However, patients without high sodium excretion had better nocturnal (p=0.024) and 24- hour BP control (p=0.036). Furthermore, there was no difference in ABPM patterns when comparing patients with high versus normal sodium excretion with hyper versus non- hyperaldosteronism. Interestingly, in the group with hyperaldosteronism, patients with normal sodium excretion had improved
dipping patterns, but only in the dipper group (p=0.016).

Conclusions
High dietary sodium intake contributes to increased nocturnal hypertension and poor 24-h BP control, but there does not seem to be a significant relationship between hyperaldosteronism and high dietary sodium intake. This data suggests that improvements in dietary sodium intake will lead to better control of nighttime BP and 24-h BP control and therefore reduces the risk of cardiovascular disease. Further studies are underway comparing these relationships in males versus females, and AAs versus Caucasians.

C. German: None.

Funding: No
Funding Component: P107

Hypertension Enhances the Differentiation of Cardiac Fibroblasts Into Myofibroblasts After TGF-beta1 Treatment

Gianluca L Perrucci, Univ of Milan, Milan, Italy; Maria Corliánò, Ctr Cardiologico Monzino, Milan, Italy; Delfina Tosi, Univ of Milan, Milan, Italy; Patrizia Nigro, Ctr Cardiologico Monzino, Milan, Italy; Gaetano Bulfamante, Univ of Milan, Milan, Italy; Giulio Pompilio, Ctr Cardiologico Monzino, Milan, Italy; Federico Lombardi, Univ of Milan, Milan, Italy

Objectives - In cardiac fibrosis associated with hypertension, TGF-beta1 plays a key role by acting on differentiation of cardiac fibroblasts (CF) into alpha-smooth muscle actin (alpha-SMA)-positive myofibroblasts. In this study, we tested the effect of TGF-beta1 during the myofibroblast differentiation process of CF from normotensive and hypertensive rats. Methods - CF were obtained by enzymatic digestion of hearts isolated from Spontaneously Hypertensive (hCF) and normotensive Wistar Kyoto (nCF) rats (n=5 rat/group). Gene and protein expression in CF was evaluated by Western blot and qRT-PCR analyses, respectively. Immunohistochemistry analysis for integrin alpha-v beta-5 was performed on rat cardiac tissue (n=5 rat/group). Results - Cultured hCF showed an enhanced SMAD2/3 activation and alpha-SMA protein expression after treatment with TGF-beta1 (5 ng/ml) in comparison with nCF. Alpha-SMA up-regulation was further confirmed by qRT-PCR analysis that showed a significant increase in alpha-SMA gene expression in hCF after TGF-beta1 treatment (2.78±0.25 vs 2.01±0.21 fold increase, p<0.05). Moreover, immunostaining on cardiac tissues revealed a higher expression of integrin alpha-v beta-5 in hypertensive vs normotensive rat hearts (345.3±170.0 vs 48.2±22.3 mm² of integrin-positive area, p<0.05). This result was also confirmed in vitro; indeed, integrin alpha-v beta-5 gene expression in hCF increased 2.8-fold in basal condition and 5.12-fold after TGF-beta1 treatment when compared to untreated nCF. Conclusions - Taken together, these results suggest that hCF are more prone to upregulate integrin alpha-v beta-5 and consequently differentiate into myofibroblasts in vitro under TGF-beta1 treatment. Thus, targeting alpha-v beta-5 might open a novel prospective for the treatment of fibrosis in hypertensive hearts likely reducing integrin-mediated TGF-beta1 activation.


Funding: No
Funding Component: P108

Circulating Matrix Metalloproteinase-2 Invades the Heart and Causes Heart Failure

Alejandro F Prado Sr., Coordination of Earth Science and Ecology, Museum Paraense Emilio
Goeldi, Belem, Brazil; Aline Azevedo, Dept of Biomechanics, Med and Rehabilitation of the Locomotor System, Ribeirao Preto Medical Sch, Univ of Sao Paulo, Ribeirao Preto, Brazil; Cibele M Prado, Dept of Pathology, Ribeirao Preto Medical Sch, Univ of Sao Paulo, Ribeirao Preto, Brazil; Larissa Pernomian, Dept of Physics and Chemistry, Faculty of Pharmaceutical Sciences from Ribeirao Preto, Univ of Sao Paulo, Ribeirao Preto, Brazil; Laena Pernomian, Dept of Physics and Chemistry, Faculty of Pharmaceutical Sciences from Ribeirao Preto, Univ of Sao Paulo, Belem, Brazil; Elen Rizzi, Dept of Pharmacology, Ribeirao Preto Medical Sch, Univ of Sao Paulo, Ribeirao Preto, Brazil; Myrella L Castro, Department of Morphology, Physiology and Basic Pathology, Faculty of Dentistry of Ribeirao Preto, Ribeirao Preto, Brazil; Simone G Ramos, Dept of Pathology, Ribeirao Preto Medical Sch, Univ of Sao Paulo, Ribeirao Preto, Brazil; Jose E Tanus-Santos, Dept of Pharmacology, Ribeirao Preto Medical Sch, Univ of Sao Paulo, Ribeirao Preto, Brazil; Raquel F Gerlach, Department of Morphology, Physiology and Basic Pathology, Faculty of Dentistry of Ribeirao Preto, Ribeirao Preto, Brazil

Background: An increase in MMP-2 levels is reported in heart failure (HF). However the role of MMP-2 in the pathogenesis of HF remains unclear. The aim of the present study was to determine the effect of increased circulating levels of MMP-2 on heart morphology and function.

Methods and Results: Purified MMP-2 catalytic domain fused to GFP (catMMP-2/GFP) or saline (control) was injected into 11-wk-old male C57BL/6 mice for four weeks. The fluorescent active protein was tracked in vivo and homed in the heart. Cardiomyocyte diameter not changed between groups (catMMP-2/GFP: 11.37 ± 0.25 μm, n=7; Control: 11.38 ± 0.13 μm, n=7; P=0.97). On the other hand, fibrosis increased in the hearts of catMMP-2/GFP mice (0.82 ± 0.05% area/field, n=7 vs Control: 0.58 ± 0.02% area/field, n=7; P<0.05). Apoptotic stained nuclei in the left ventricle (LV) of catMMP-2/GFP injected-mice amounted to 7.24%, n=4, P<0.05, whilst the LV of control animals only exhibited 0.27%, n=4. catMMP-2/GFP localized in the heart interstitium, where increased proteolytic activity (15.13 ± 1.33 U, n=5 vs Control: 9.70 ± 0.42 U, n=5; P<0.05). Hearts of catMMP-2/GFP mice showed 25% decrease in cardiac output (13 ± 1 mL/min, n=9 vs Control: 17 ± 1 mL/min, n=9), 30% decrease in ejection fraction (40 ± 2 %, n=9 vs Control: 56 ± 2 %, n=9) and stroke volume (28 ± 2 μL, n=9 vs Control: 40 ± 2 μL, n=9), and 34% decrease in fractional shortening (18 ± 1 %, n=9 vs Control: 28 ± 1 %, n=9) (P<0.05 for all data). Western blotting showed 40% decrease in N-cadherin in animals that received catMMP-2/GFP (0.53 ± 0.08 U, n=6 vs control: 0.89 ± 0.11 U, n=6; P<0.05). Expression of signaling proteins also changed in LV of catMMP-2/GFP mice: TGF-β1 expression increased by 30% (0.60 ± 0.07 U, n=7 vs control: 0.40 ± 0.05 U, n=7, P<0.05), pAkt/Akt decreased by 40% (0.46 ± 0.05 U, n=7 vs control: 0.76 ± 0.11 U, n=7; P<0.05), pSMAD2/total SMAD2 ratio increased (1.88 ± 0.22 U, n=6 vs control: 0.93±0.12 U, n=6, P<0.05), and pSMAD3/total SMAD3 ratio increased (1.24 ± 0.22 U, n=7 vs control: 0.33 ± 0.07 U, n=7; P<0.05).

Conclusions: Circulating active MMP-2 homing to the heart interstitium degrades N-cadherin and induces TGF-β1 overexpression, leading to apoptosis, and fibrosis. This mechanism may account for heart function loss when plasma levels of MMP-2 increase.


Funding: No
Spiny Mice Are Protected From Myocardial Infarction Induced Cardiac Pathophysiology

YanFei Qi, Univ of Florida, Gainesville, FL; Juan Zhang, The First Affiliated Hosp of Soochow Univ, Soochow city, China; Lei Wang, Ashok Kumar, Avinash Mandloi, Ravneet Vohra, Glenn A Walter, Joshua F Yarrow, Dipankar Gupta, Michael J Katovich, Mohan K Raizada, Carl J Pepine, Univ of Florida, Gainesville, FL

Background: Despite the advancement in drug and surgical interventions, myocardial damage and associated cardiac dysfunction lead to heart failure that remains common cause of death following myocardial infarction (MI). Spiny mice (Acomys cahirinus, SM) have been shown to possess regenerating capacity following deep tissue injury without scarring (Nature 2013). This led us to investigate if this regenerative property would also be preserved in the heart.

Methods and Results: Adult CD1 and SM were subject to left anterior descending coronary artery ligation or sham surgeries. Proliferative cells were identified by nuclear incorporation of 5-bromodeoxyuridine (BrdU, daily, i.p.) and injection was started from 3d post MI continued to 2wks post MI. Cardiac function was assessed using echocardiography and MRI. SM exhibited 3-fold smaller infarct size (SM-MI 18.6±3.4% vs CD1-MI 76.2±3.4%, p<0.05) and better contractility measured by ejection fraction (SM-MI 77.1±6.5 vs CD1-MI 24.6±4.6, %, p<0.05) than CD1 mice. SM showed 6-fold increase in BrdU+ cells in left ventricle after MI while CD1 mice had 4-fold increase (CD1-sham 11±3.5 vs CD1-MI 44±9.1 and SM-sham 16±9.8 vs SM-MI 101.1±30.9, p<0.05). Though basal cardiac ACE2 activity was not different between CD1 and SM, MI resulted in a 16% decrease in cardiac ACE2 activity in CD1-MI mice but 20% elevation of cardiac ACE2 activity in myocardial tissue in SM-MI.

Conclusions: SM are protected from ischemia induced cardiac damage and dysfunction. This involves increased proliferating cardiac cells and reduction in infarct size. Thus SM could be an ideal animal model for identification of molecular and genetic circuits involved in preservation/regeneration of cardiac function with translational implication to human MI.

Y. Qi: None. J. Zhang: None. L. Wang: None. A. Kumar: None. A. Mandloi: None. R. Vohra: None. G.A. Walter: None. J.F. Yarrow: None. D. Gupta: None. M.J. Katovich: None. M.K. Raizada: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; R01 HL056921, R01 HL132448. C.J. Pepine: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5UM1HL087366-09, 2 UM1 HL087366-06, UL1 TR000064.

Funding: No

Low Dose Minocycline Lowers BP and Improves Inflammatory Status in Patients with Treatment Resistant Hypertension

YanFei Qi, Seungbum Kim, Dana D Leach, Sarah J Long, Eileen Mary Handberg, Vermali Rodriguez, Avinash Mandloi, Mohan K Raizada, Carl J Pepine, Univ of Florida, Gainesville, FL

Background: About 15% of hypertensive patients have treatment resistant hypertension (TRH), which substantially increases mortality risk. Based on our previous studies and evidence from literature, we have proposed that systemic and neuro-inflammation is crucial in development and establishment of TRH. This study was to test the hypothesis that minocycline (Mino), an anti-inflammatory antibiotic, would lower BP and inflammatory
markers in patient with TRH. Selection of Mino was based on our preliminary studies showing that Mino can inhibit systemic inflammation and penetrate the blood brain barrier to attenuate neuroinflammation in addition to its antibiotic effects. **Methods:** A total of 29 subjects was recruited for this study. Exclusion and inclusion criteria are described at Clinicaltrials.gov (NCT02133872). Office and ambulatory BP measurements (ABPM) were used to confirm TRH diagnosis and subsequently measured 30d and 60 d after Mino administration (50mg/d). If BP failed to decline after 60 d Mino treatment (50mg/d), Mino was increased to 100mg/d and patients were monitored at 60 d. Circulating high-sensitivity C-reactive protein (hs-CRP), high-mobility group box 1 (HMGB1), and Th17 levels were measured. **Results:** Average BP of TRH patients was 149.5/78.5mmHg, 67% responded to 50mg/d Mino and 33% to 100mg/d. ABPM 24h average BP for all m-TRH was <136/69mmHg 60 d after Mino, and ABPM showed systolic and diastolic BP were significantly reduced by 14.5/10.5 mmHg. Plasma HMGB1 levels were 8- and 5-fold higher in TRH than normotensive (N) and controlled hypertensive (CH) patients, respectively (TRH: 45.2±25.10 vs N: 5.5±3.8 and CH: 8.9±10.2, ng/ml, p<0.05). Mino reduced HMGB1 level in TRH (m-TRH 3.0±0.7, ng/ml p<0.05). Furthermore, there were 3-4-fold increases in Th17 cells and 67% increase in hs-CRP levels in TRH patients compared to N and CH. Mino treatment resulted in significant reduction in these parameters and did not change BMI in TRH patients. **Conclusions:** This study demonstrates that a low dose Mino has a significant antihypertensive effect and lowers inflammatory markers in TRH patients. Thus, Mino could be considered a promising therapeutic option for TRH patients.

**Y. Qi:** None. **S. Kim:** None. **D.D. Leach:** None. **S.J.B. Long:** None. **E.M. Handberg:** None. **V. Rodriguez:** None. **A. Mandloi:** None. **M.K. Raizada:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; R01 HL056921. **C.J. Pepine:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5UM1HL087366-09, 2 UM1 HL087366-06, UL1 TR000064.

**Funding:** No

**Funding Component:** P111

**Inhibition of Mitochondrial Fission Enhances Cardiac Angiogenic Responses After Ischemic Reperfusion Injury: Implications for Post-transcriptional Regulatory Mechanisms**

**Sudhakar Veeranki,** Suresh C Tyagi, Univ of Louisville, Louisville, KY

**Aims/hypothesis:** Although the mitochondrial fission inhibitor, Mdivi-1, has been shown to ameliorate I/R injury, the modulations in angiogenic program after myocardial I/R injury are unknown. We hypothesized that Mdivi-1 treatment confers positive angiogenic responses after I/R injury, thereby improving the repair process. **Methods:** Female mice were treated with Mdivi-1 inhibitor for a period of 5 days (50mg/kg, I.P., daily). Both control and treatment groups underwent 30 min of ischemia and 72 hrs of reperfusion. On the day 3 of I/R injury, mice were sacrificed and the ischemic and non-ischemic portions of heart tissue were collected. Relative levels of 53 angiogenesis-related proteins were quantified simultaneously by using Angiogenic arrays. Heart function was evaluated before and after 72 hrs of I/R injury. Changes in the protein levels were verified by Q-PCR. Global methylation levels were enumerated to test the extent of methylation changes after I/R injury and after Mdivi-1 treatment. **Results:** Mdivi-1...
treatment ameliorated I/R induced changes in the fractional shortening, ejection fraction and LV inner diameter. Although, several of key markers of myocardial infarction or I/R injury were clearly upregulated in the ischemic tissues of both the groups, there are clear differences in the relative levels of certain key angiogenic regulators. Notably, the levels of pro-angiogenic factors such as IGFBP1 (168% vs 216%) and FGF1 (154% vs 177%) were significantly elevated with the exception of Angiopoietin1 (212% vs 158%), while the anti-angiogenic factors such as ADAMTS1 (153% vs 111%) and MMP3 (218% vs 183%) were significantly suppressed. The changes in the protein levels were not entirely matched by the changes in the mRNA levels, suggesting a role for post-transcriptional regulatory mechanisms.

**Conclusions:** This is the first report on the role of mitochondrial dynamics (mitophagy) in regulation of myocardial I/R induced angiogenic responses. Overall, inhibition of excessive mitochondrial fission after I/R injury ameliorated heart dysfunction and enhances injury repair through positive angiogenic profile. The findings will aid in shaping rationalized drug development for prevention of ischemic heart failure.

**S. Veeranki:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Data belongs to a grant under revision. **S.C. Tyagi:** None.

Funding: No

Funding Component: P112

**Decreased α-Myosin Expression is Associated with Left Ventricular Hypertrophy in Hypertensive Caribbean Vervets**

**Chelsea C Weaver,** Anthony Gutierrez, Jeffrey L Osborn, Univ of Kentucky, Lexington, KY

Caribbean vervets (*Chlorocebus aethiops sabeus*) develop hypertension (HT; Systolic Blood Pressure ≥ 140 mmHg) in over 30% (125/345) of the outbred population. Elevated total peripheral resistance in HT increases cardiac afterload, which may lead to left ventricular hypertrophy (LVH) and cardiac remodeling. We hypothesize that prolonged spontaneous HT is associated with LVH, cardiac and aortic fibrosis, as well as differential transcription of myocardial contractile proteins. Vervets were characterized as HT (SBP = 170 ± 25.3 mmHg; n=125) or normotensive (NT, SBP = 99 ± 14.5 mmHg; n=148) using forearm plethysmography (ketamine sedated;15 mg/kg i.m.). Cardiomyocyte cross-sectional area was greater in HT compared to NT animals (HT 283 ± 52 µm², n=9 vs NT 114 ± 8 µm², n=10; p<0.01). Average collagen stained as a function of tissue area was similar in left ventricular myocardium of HT and NT animals (HT 14.17 ± 3.13% or 0.17/1.21 mm², n=9 vs NT 12.22 ± 0.80% or 0.16/1.27 mm², n=10; p>0.05). Aortic adventia collagen area was greater in HT compared to NT vervets (HT 66.12 ± 4.22% or 0.60/0.90 mm², n=6 vs NT 54.53 ± 2.21% or 0.56/1.02 mm², n=10; p<0.05). Total tissue collagen was estimated using a hydroxyproline assay. Collagen content was not different between HT and NT vervets for left ventricular myocardium (HT 194.02 ± 8.61 µg/mL, n=11 vs NT 201.70 ± 18.89 µg/mL, n=10; p=0.71) or aorta (HT 745.64 ± 44.49 µg/mL, n=11 vs NT 668.39 ± 31.06 µg/mL, n=11; p=0.17). Myosin gene expression (α and β) was estimated using RT-PCR of mRNA in left ventricular myocardium of NT (SBP = 98.91 ±10.89 mmHg; n=20) and HT (SBP = 171.51 ± 30.28 mmHg; n=17) vervets. α-myosin was downregulated in HT compared to NT vervets (HT RQ = 0.10 ± 0.03 vs. NT RQ = 0.22 ± 0.04; p<0.05), while β-myosin expression was not different (HT RQ = 0.22 ± 0.17 vs. NT RQ = 0.20 ± 0.16; p=0.83). Thus, spontaneous HT in outbred vervets induces LVH in response to factors other than cardiac fibrosis. Myosin gene
expression may shift from α-myosin to other contractile protein isoforms, characteristic of human heart failure. In this nonhuman primate model, HT does not induce significant aortic fibrosis that may occur in aged animals. Future studies will further characterize contractile and pro-inflammatory proteins in LVH of spontaneous HT vervets.

**C.C. Weaver:** None. **A. Gutierrez:** None. **J.L. Osborn:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectual property) Significant; BSRG, LLC. Funding: No Funding Component: P114

**Cardiac and Renal Adrenergic Receptor Expression in Spontaneously Hypertensive Caribbean Vervets**

Megan K Rhoads, Jeffrey L. Osborn, Univ of Kentucky, Lexington, KY

Sympathetic nerve activity and adrenergic receptor stimulation causes development and maintenance of hypertension in patients and experimental animal models. The Caribbean vervet (Chlorocebus aethiops sabaues), an Old World non-human primate, is a translational model of human cardiovascular disease due to its recent divergence, genetic similarity, upright posture, complex social structure, and diurnal behavior. Outbred and randomly selected adult Caribbean vervets exhibit systolic blood pressures (SBP) >140 mmHg in 36% of our cohort (124 of 345 individuals). We hypothesized that gene expression of adrenergic receptor subtypes in renal and cardiac tissue would be elevated in HT Caribbean vervets, and may contribute to spontaneous hypertension. Gene expression was measured using qRT-PCR and RQs compared using the Mann-Whitney U Test. HT animals (n=18, SBP: 168.24±7.25mmHg) had increased expression of both α- and β-adrenergic receptor subtypes in renal outer medulla (α1a: NT RQ 1 ± 0.43 vs. HT RQ 2.26 ± 0.50, p<0.05; α1d: NT RQ 1 ± 0.34 vs. HT RQ 2.25 ± 0.32, p<0.05; α2a: NT RQ 1 ± 0.24 vs. HT RQ 1.92 ± 0.19, p<0.05; α2c: NT RQ 1 ± 0.15 vs HT RQ 1.65 ± 0.15, p<0.05; β1: NT RQ 1 ± 0.24 vs. HT RQ 1.88 ± 0.18, p<0.05; and β2: NT RQ 1 ± 0.21 vs. HT RQ 1.87 ± 0.24, p<0.05) compared to NT animals (n=18, SBP: 96.61±3.2 mmHg). All α- and β1-adrenergic receptor expression in renal cortex and inner medulla was similar between the two groups. Inner medullary β2 adrenergic receptor expression was elevated in HT animals (NT RQ 1 ± 0.25 vs HT RQ 1.62 ± 0.10, p<0.05). In the heart, β1 adrenergic receptor expression was upregulated in the left ventricular myocardium of HT animals (NT RQ 1 ± 0.16 vs HT RQ 1.88 ± 0.35). Together, these data suggest that the HT Caribbean vervet may exhibit an exaggerated response to sympathetic nerve activity due to increased adrenergic receptor expression in the kidney and heart, which could contribute to the spontaneous hypertension observed in this translational large animal model.

M.K. Rhoads: None. **J.L. Osborn:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectual property) Significant; BSRG, LLC. Funding: No Funding Component: P116

**Exercise Ameliorated Cognitive Impairment Through ER Stress Mitigation in Alcohol Treated Exercise Ameliorated Cognitive**
Impairment Through Er Stress Mitigation in Alcohol Treated C57bl/6 Male Mice

Akash George, Kennedy Richardson, Yuankun Zhai, Suresh C Tyagi, Neetu Tyagi, Univ of Louisville, Louisville, KY

Alcohol consumption is a potent inducer of oxidative stress (OS). Oxidative stress cause disturbance of endoplasmic reticulum (ER) homeostasis, that triggers ER stress (ERS), cause neuronal damage in the brain. Our Previous data indicate that Alcohol consumption induces mitochondrial dysfunction and free radical production in mouse cerebral cortex. Exercise has been recommended by clinicians as a secondary protective therapy; however, its effect on brain functions through ER stress has not been fully explored. Therefore, we hypothesized that exercise improves Alcohol-induced neurodegeneration and decline in cognitive function through ER stress mitigation. To test this hypothesis, we selected 10-12 weeks old male wild-type mice (C57BL/6, WT), grouped as follows: 1) WT, 2) WT+ Alcohol, 3) WT+ Exercise, 4) WT+ Alcohol + Exercise. Mice were given an intraperitoneal injection of Alcohol (1.5g/kg BW) or saline solution every day for 8 weeks. The mice were exercised for 8 weeks on a treadmill with a controlled speed of 7 meters/min for the first week, the speed of 10 meters/min for the second week and 11 meters/min in the following weeks and a total of 330 meters every day. The mice were given rest of 10 minutes. Cognitive and behavior alterations were assessed by novel object recognition, Passive avoidance, and Y-maze tests. Our result showed there is a significantly impaired cognitive and behavior functions (600.00 ± 0.00 vs 480 ± 20.00, P<0.05) in Alcohol-treated group compared to WT control mice. However exercised significantly improved (0.37 ± 0.05 vs 0.63 ± 0.04 P<0.01) these functions as compared to Alcohol-treated group. Also, we observed an elevated blood pressure in the Alcohol-treated group (123.50 ± 1.17) and exercise brought that to the normal level (108.98 ± 4.47, P<0.01). In addition, the effect of exercise on neuronal survival in the Alcohol-treated mouse brain was confirmed by a decrease in by fluoro-jade C reactivity. Taken together, our results indicate a myriad of beneficial effects of exercise over ER mitigation in Alcohol-treated mice. Furthermore, our findings suggest exercise alleviates neurodegeneration and cognitive dysfunction and thereby improving total brain function. This work was supported by NIH grant HL107640-NT

A. George: None. K. Richardson: None. Y. Zhai: None. S.C. Tyagi: None. N. Tyagi: None.

Funding: No

Funding Component: P117

A Conserved Trans-membrane Domain Lysine in the Integrin Beta1 Subunit Regulates Renal Collecting Duct Cell Function

Sijo Mathew, Vanderbilt Univ Medical Ctr, Nashville, TN; Jiang Chen, Zhenwei Lu, Charles Sanders, Vanderbilt Univ, Nashville, TN; Roy Zent, Vanderbilt Univ Medical Ctr, Nashville, TN

Integrins are heterodimeric trans-membrane receptor proteins that mediate the interaction of cells with extracellular matrix proteins (ECM). They mediate various growth factor dependent cell-signaling pathways during the development of fibrosis that is characteristic of all forms of chronic kidney diseases. Although integrin β1 is the most abundant integrin subunit in kidney and can form complexes with 12 different α subunits, integrin β3 is the best studied integrin β subunit and serves as the canonical model for integrin function based on the high sequence homology between the trans-membrane and cytosolic domains of integrin β subunits. A
conserved lysine residue towards the C-terminus of integrin β3 subunit is reported to be important for regulating the activation of integrin αIIbβ3 complexes; however the functional importance of this lysine is unknown in β1 integrins. We investigated the role of this lysine residue in integrin β1-dependent kidney collecting duct cell function. We expressed the mutant protein where the lysine is mutated to glutamic acid in collecting duct cells null for integrin β1. Collecting duct cells expressing mutant protein had decreased the adhesion of cells to collagen IV mediated by integrin α1β1 by 80% (0.95 vs 0.18). This mutation also decreased the ability of IMCD cells adhesion to collagen I mediated by integrin α2β1 by 82% (0.78 vs 0.15). In contrast to earlier reports in integrin β3, this mutation did not significantly alter the amount of active integrin β1 on the cell surface as estimated by FACS analysis; however we did observe a decrease in conformation specific antibody binding on cells adhered to collagen (0.70 vs 0.30). We also investigated the role of this lysine residue in complex formation of purified integrin β1 with integrin α1 and α2 TM/CT domains in phospholipid bicelles using fluorescence anisotropy. The dissociation constant for binding was estimated to be >3.2 mol and mutation of lysine residue did not significantly alter their binding ability. This contrasted with integrin αIIb β3 where we found fourfold decrease in binding ability (Kd 0.09 ± 0.03 mol and 0.33 ± 0.05 mol). Our data clearly suggest that conserved transmembrane lysine in both integrin β3 and integrin β1 regulate cell functions by distinct mechanisms.

S. Mathew: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH RO1. R. Zent: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH RO1.

Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P118

The Complexity of the Albuminuria Development in Salt-sensitive Hypertension

Bradley T Endres, Medical Coll of Wisconsin, Milwaukee, WI; Ruben M Sandoval, George J Rhodes, Silvia B Campos-Bilderback, Malgorzata M Kamocka, Indiana Univ Sch of Med, Indianapolis, IN; Christopher McDermott-Roe, Alexander Staruschenko, Medical Coll of Wisconsin, Milwaukee, WI; Bruce A Molitoris, Indiana Univ Sch of Med, Indianapolis, IN; Aron M Geurts, Oleg Palygin, Medical Coll of Wisconsin, Milwaukee, WI

Hypertension is one of the most prevalent diseases worldwide, and is a major risk factor for developing albuminuria, renal failure and cardiovascular diseases. The goal of this study was to investigate the mechanisms leading to albuminuria in the kidney of a canonical rat model of hypertension, the Dahl salt-sensitive (SS) rat. To determine the relative contributions of the glomerulus and proximal tubule (PT) to albuminuria, we applied intravital two-photon-based imaging to investigate complex changes in renal function that occur during salt-induced hypertension. Following a high salt (HS) diet, SS rats exhibited elevated blood pressure, accompanied by increased glomerular sieving of albumin (GSC_{albumin}=0.0686) and decreased serum albumin (Δ0.54 g/dL), which corresponded to increased daily filtered albumin up to 3.7 vs 0.8
g at normal diet. Pathologically, hypertensive animals had significant tubular damage as indicated by increased prevalence of granular casts (+Δ2.20 casts/image), dilation/expansion and necrosis of PT epithelial cells, increased vascular injury (+Δ0.61 leakage/image), and progressive inflammation. HS diet significantly reduced total PT uptake of albumin, which also coincided with reduced transcellular transport of albumin back into circulation. Collectively, these results indicate that both the glomerulus and the proximal tubule contribute to albuminuria and dual treatment of glomerular filtration and albumin reabsorption may represent an effective treatment of salt-sensitive hypertension.


Funding: No

Funding Component: P119

Association Between Inspiratory Muscle Strength and Sympathetic Cardiac Modulation and Baroreflex in Patients With End Stage Renal Disease Undergoing Hemodialysis

Kátia B Scapini, Silvia B Souza, Valéria C Hong, Oscar A Moraes, Jacqueline F Machi, Heart Inst (InCor), Univ of São Paulo, Medical Sch, São Paulo, Brazil; Cristiano T Mostarda, Federal Univ of Maranhão, São Luiz, Brazil; Fernanda M Consolim-Colombo, Maria C Irigoyen, Heart Inst (InCor), Univ of São Paulo, Medical Sch, São Paulo, Brazil

Introduction: Patients with end-stage renal disease (ESRD) are susceptible to development of autonomic dysfunction and have metabolic and muscular changes which are associated with decreased functional capacity. Objectives: To evaluate respiratory muscle strength and cardiac autonomic modulation in ESRD patients undergoing hemodialysis. Methods: ESRD patients undergoing hemodialysis (n= 9, mean age 46.2±14.1 years) and healthy controls (n =7, mean age 44.6±14.6 years) were assessed. Non–invasive curves of blood pressure were recorded continuously (Finometer ®) for 10 minutes. The heart rate variability was estimated in the time and frequency domain. Inspiratory and expiratory maximal pressures (IPmax and EPmax) were quantified using a pressure transducer. Mann-Whitney and Spearman tests were used. The results were expressed as mean ± SD. Results: Regarding respiratory muscle strength, ESRD patients had lower inspiratory (IPmax: 79.1±25.0 vs 121.3±24.3cmH2O, p=0.03) and expiratory muscle strength (EPmax 92.8±23.1 vs 153.8±23.5cmH2O, p=0.01) then healthy controls. ESRD patients presented lower heart rate variability than healthy controls (SDNN: 19.2±4.0 vs 52.6±18.8ms, p=0.000) and lower RMSSD, an index of vagal modulation (11.8±5.2 vs 46.1±20.4ms, p=0.000). Regarding frequency domain indexes, ESRD patients presented higher low-frequency component (LFnu= 68.9±11.9 vs 47.3± 2.8, p=0.008), lower high-frequency component (HFnu= 31.1±11.9 vs 52.7±12.8, p=0.008) and higher sympathovagal balance (LF/HF: (LF/HF= 3.1±1.3 vs 1.1±0.5, p=0.005) when compared to controls. Furthermore baroreflex sensitivity was reduced in ESRD patients (alfa index: 3.6±1.6 vs 11.7±4.4ms/mmHg, p=0.002). In ESRD patients the IPmax was significantly associated with LF (r=-0.813, p=0.014) and with alfa index (r=-0.900, p=0.037). Conclusion: ESRD patients undergoing hemodialysis presented reduced inspiratory muscle strength, increased sympathetic modulation and reduced cardiac vagal modulation and impaired baroreflex sensitivity. In these patients inspiratory muscle
strength was associated with sympathetic cardiac modulation and baroreflex.


Funding: No
Funding Component: P120

Exercise in Patients With Chronic Kidney Disease on Hemodialysis: Systematic Review and Network Meta-analysis of Randomized Clinical Trials

Kátia B Scapini, Oscar A Moraes, Heart Inst (InCor), Univ of São Paulo, Medical Sch, São Paulo, Brazil; Graciele Sbruzzi, Univ Federal do Rio Grande do Sul, Escola Superior de Educação Física, Dept de Educação Física., Porto Alegre, Brazil; José F Inácio, Insto de Cardiologia do Rio Grande do Sul, Porto Alegre, Brazil; Clarissa G Rodrigues, Insto de Cardiologia do RS / Fundação Universitária de Cardiologia, IC/FUC, Programa de Pós-Graduação, Porto Alegre, Brazil; Camila P Leguisamo, Univ de Passo Fundo, FEFF, Passo Fundo, Brazil; Hugo Tourinho Filho, Univ de São Paulo, Escola de Educação Física e Esporte (EEFERP), Ribeirão Preto, Brazil; Maristela Böhke, Univ Católica de Pelotas, Hosp Universitário São Francisco de Paula, Pelotas, Brazil; Maria C Irigoyen, Heart Inst (InCor), Univ of São Paulo, Medical Sch, São Paulo, Brazil

Background: Exercise provides beneficial effects in ESRD, however few studies compare efficacy of different modalities of exercise in this population. To overcome the restrictions of limited available comparisons we employed network meta-analysis of randomized clinical trials (RCTs) to compare the efficacy of different modalities of exercise on aerobic capacity, arterial blood pressure and hemodialysis efficacy in ESRD adults patients on hemodialysis treatment. Methods and Findings: We searched PubMed, EMBASE, COCHRANE CENTRAL, WEB OF SCIENCE and LILACS databases for RTCs with adults ESRD patients on hemodialysis that compared aerobic exercise (AE), resistance training (RT) or combined training (CT) with a control group. Were included 28 trials involving 1045 ESRD patients on hemodialysis. Direct meta-analysis show that both, AE (3.35, 95% CI: 1.79, 4.91 ml/kg/min) and CT (5.0, 95% CI: 3.50, 6.50 ml/kg/min) improved aerobic capacity while only CT reduced systolic (-5.88, 95% CI: -9.83, -1.93 mmHg) and diastolic (-3.93, 95% CI: -6.23, -1.60 mmHg) arterial blood pressure. Regarding hemodialysis efficacy, only AE was able to improve Kt/V (0.16, 0.05, 0.26). Network meta-analysis showed that AE and CT was superior to control group for aerobic capacity and CT presented 92.8% of probability to be ranked as first most effective treatment. For arterial blood pressure, only CT was superior to control group and for diastolic blood pressure combined training was also significantly superior to AE. Moreover, CT presented 94.7% probability of being best treatment for systolic arterial pressure and 98.7% for diastolic arterial pressure. None modality of exercise was superior to control treatment for hemodialysis efficacy.

Conclusions: The main contribution of this network meta-analysis is to ranking the potential benefits of different exercise modalities on health outcomes in hemodialysis patients. The analysis found that combined training, aerobic plus resistance training, is the most effective modality to increase aerobic capacity, and blood pressure control, in hemodialysis patients. This finding comes to fill the gap created by the lack of head-to-head comparison of different modalities of exercise in chronic kidney disease patients.

K.B. Scapini: None. O.A. Moraes: None. G. Sbruzzi: None. J.F.S. Inácio: None. C.G.
Antenatal Corticosteroids and Alterations in Renal Function in Adolescents Born Preterm


Background: Treatment with antenatal corticosteroids (ANCS) hastens fetal lung development and improves survival of infants born preterm, but the long-term effects of ANCS are not well described. Animal models suggest ANCS causes programmed alterations in renal maturation, including a decreased number of functional nephrons. Data in humans suggests that prematurity is associated with subsequent development of glomerular hyperfiltration and albuminuria as precursors of chronic kidney disease. We hypothesized that ANCS exposure alters renal function in adolescents born preterm. Methods: A cohort of 169 adolescents born prematurely, 91 of whom were exposed to ANCS, was evaluated at age 14. We measured plasma and urine creatinine (Cr) and urine albumin and calculated the estimated glomerular filtration rate (eGFR) and urine albumin-to-Cr ratio (ACR). The outcomes were albuminuria (urine ACR >30 mg/g Cr) and hyperfiltration (eGFR ≥147.5 mL/min/1.73 m², the upper quintile of the cohort’s eGFR). We used multivariable logistic regression models to evaluate the association of ANCS exposure with renal outcomes, adjusting for confounding variables. Results: In unadjusted analyses and after adjustment for race, sex, and maternal hypertension, ANCS exposure was associated with a decreased likelihood of albuminuria (adjusted OR 0.33, 95% CI 0.12 to 0.91, p = 0.03). In unadjusted analysis ANCS was associated with an increased likelihood of hyperfiltration (unadjusted OR 3.17, 1.1 to 9.15, p = 0.03), and this relationship was only slightly attenuated after adjustment for confounding variables (p = 0.12).

Conclusions: Adolescents born prematurely who were exposed to ANCS were less likely to exhibit albuminuria, but were more likely to have hyperfiltration. ANCS could affect renal function among adolescents born prematurely.

A.M. South: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Co-investigator on P01 grant Prenatal Events-Postnatal Consequences. E. Honoraria; Modest; Alexion Pharmaceuticals. G. Consultant/Advisory Board; Modest; Alexion Pharmaceuticals. P.A. Nixon: None. M.C. Chappell: None. D.I. Diz: None. G.B. Russell: None. B.M. Snively: None. H.A. Shaltout: None. J.C. Rose: None. T.M. O'Shea: None. L.K. Washburn: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; P01 Prenatal Events-Postnatal Consequences.

Funding: No

Funding Component: P122

Higher Systemic Vascular Resistance Impairs Hypertension Control

Bashar S Amr, Albert Botchway, Lowell Hedquist, John M Flack, Southern Illinois Univ, Springfield, IL

Background: Systemic vascular resistance (SVR) elevation is the hallmark pathophysiological
abnormality in established essential hypertension (HTN). We hypothesized that higher baseline SVR would reduce the likelihood of HTN control over follow-up amongst hypertensive patients.

**Methods:** We undertook a retrospective cohort study (N= 281) using de-identified data from patients evaluated in an urban hypertension clinic between November 1998 and May 2010. Patients were included if they had an established diagnosis of HTN as well as impedance cardiography (ICG) non-invasive hemodynamic testing, as well as 2 follow up clinic visits over a minimum of 8 weeks after the ICG procedure; follow-up was truncated at 1-year post-ICG measurement. The cohort was stratified into three SVR tertiles (I [< 1336], II [1340 - 1836] and III >1868), reference category. Kaplan-Meier survival curve analysis was used to test for differences in time to first HTN control across SVR tertiles. We examined the first occurrence of HTN control (cox proportional hazards models) and HTN control rates over follow-up (cumulative hazards models) according to SVR tertile; both hazards models were adjusted for age, diabetes status, eGFR, and race.

**Results:** The proportion of individuals in the SVR tertiles I, II and III (highest, reference) ever attaining new HTN control during follow-up were 72.3%, 71.2% and 58.1%; Kaplan-Meier analyses showed heterogeneity in the proportions ever attaining control across SVR tertiles (P=0.0113). The number of days to 50% attainment of HTN control in SVR tertiles I, II and III were 106, 103, and 162. Relative to the highest SVR tertile (III),

**Conclusion:** SVR is a physiological marker for pharmacological treatment resistance in actively managed patients with hypertension as the probability of HTN control relates inversely to non-invasively estimated SVR.

B.S. Amr: None. A. Botchway: None. L. Hedquist: None. J.M. Flack: G. Consultant/Advisory Board; Modest; The Medicine Company, Medtronic, BackBeat Medical Inc, Bayer, Forset Laboratories Inc.

Funding: No

Prevalence of Angiotensin Converting Enzyme Inhibitor-induced Cough and Impact on the Quality of Life of Hypertensive Patients in a Tertiary Health Institution in Nigeria: A Pilot Study

**Background/Objective.** Angiotensin Converting Enzyme (ACE) inhibitors have widespread use and have been shown to be cardio- and renoprotective. However they have a class side effect of dry persistent cough that has been shown to have a significant impact on the physical, social and psychological health of patients. This study was carried out to determine the prevalence of ACE inhibitor-induced cough and to assess its impact on the quality of life of hypertensive patients in Lagos University Teaching Hospital (LUTH), Nigeria.

**Method.** The study was centered at the outpatient cardiology clinic of LUTH. It was a cross sectional descriptive study with the use of interviewer-administered validated structured questionnaires. The Leicester Cough Questionnaire (LCQ) was used to assess the effect of the cough on the patients quality of life while the Visual Analogue Scale assessed relative to the highest SVR tertile (III).
the severity of the cough. Ethical clearance was obtained from the Research and Ethics committee of LUTH and patients consent sought prior commencement of the study. The data obtained was analyzed using SPSS version 20.0 and represented as simple percentages and frequencies. Bivariate analysis was carried out using chi square test. A p-value <0.05 was considered statistically significant.

**Result.** Out of the 101 patients interviewed, 20 patients had experienced ACE inhibitor-induced cough giving a prevalence of 19.8%. The prevalence of the cough was also found to be higher in females (13.86%, 14 of 20) than in males (5.94%, 6 of 20). An LCQ mean score of 13.895 was obtained which showed an average quality of life of the patients. The cough symptom severity results also showed that 40% (8 of 20) of the patients had a mild cough, 35% (7 of 20) had a moderate cough and 25% (5 of 20) had a severe cough.

**Conclusion.** Dry cough is a relatively common side effect associated with ACE inhibitor therapy. The cough induced had an average impact on quality of life and was not severe enough to lead to discontinuation of the ACE inhibitor therapy in most patients.

C.E. Eze: None. A.D. Oloidi: None.

Funding: No

Funding Component: P124

**“Beyond Silos” Model of Homecare Improves Blood Pressure Control in Multimorbid Hypertensive Patients**

Antonietta Valeria Pascale, Univ of Salerno, Baronissi, Italy; Rosa Finelli MD, AOU San Giovanni e Ruggi, Univ of Salerno, Baronissi, Italy; Rocco Giannotti, Valeria Visco, Ida Matula, Univ of Salerno, Baronissi, Italy; Giuseppe Vairo, Magadi Home, Salerno, Italy; Michele Ciccarelli, Univ of Salerno, Baronissi, Italy; Enrico Coscioni, AOU San Giovanni e Ruggi, Univ of Salerno, Salerno, Italy; Guido Iaccarino, Univ of Salerno, Baronissi, Italy

**INTRODUCTION:** Less than 40% of hypertensive on antihypertensive treatment have pressure <140/90 mmHg. Among the most common causes is poor adherence to therapy, in particular in multimorbid, polytreated patients. ICT based new models of care like Smartcare or Beyond Silos (www.beyonsilos.eu) that offer patient-centered home care including telemonitoring, represent a new tool to strengthen the doctor-patient interaction, and improve compliance of chronic patients.

**METHODS:** To verify the impact of home care strategy to improve pressure control, from February 1 to March 31 2016 we selected patients who accessed the Outpatient Clinic for Hypertension at the AOU San Giovanni e Ruggi in Salerno, those with 1) poor BP control >140 and/or DBP>90 mmHg) after at least three follow-up visits in the last year, 2) optimal drug therapy, 3) poor adherence to therapy, 4) at least one concurrently treated chronic condition. Patients who signed informed consent received “Beyond Silos” home care program, including a weekly nurse access, for four weeks, and telemonitoring through 3G-connected devices of systolic (SBP) and diastolic (DBP) blood pressure, heart rate (HR) and body weight measuring and Oxygen Saturation. Each patient was instructed to scheduled self- assesment of the above parameters that were stored on the Local Health Authority server of Salerno. Treatment compliance was verified weekly by the nurse through drug blister count. After 4 weeks, patients were evaluated at the hospital premises. **RESULTS:** We selected seven patients (M/F=5/2; age 73.4 ± 2.3 years). In this population BP control that went from ambulatory SBP/DBP 155±5/74±4mmHg to 111±1.7/95.9±2.7mmHg, (p<0.001). Adherence scores assessed at the home of the patients also were improved. No changes were observed in
HR, weight and oxygen saturation.

**CONCLUSION:** Our data show that patients with a history of loose BP control despite optimal therapy can achieve controlled BP through Beyond Silos home care program within a month. This suggests that strategies of ICT based home care might represent a real breakthrough in the management of chronic conditions, in particular for multimorbid, poor compliant patients. Future large scale studies are needed for assessing long term effects on cardiovascular outcome.


Funding: No

Funding Component: P125

**Performance of Ambulatory Blood Pressure Monitoring in Predicting Autonomic Dysfunction**

Abhilash Koratala, Kawther F Alquadan, Abutaleb A Ejaz, Univ of Florida, Gainesville, FL

**BACKGROUND:** We investigated the predictive performance of orthostatic hypotension (OH) and ambulatory blood pressure monitoring (ABP) to predict autonomic dysfunction.

**METHODS:** In this retrospective analysis, statistical associations among the candidate variables were investigated and comparisons of predictive performances were performed using Receiver Operating Characteristics (ROC) curves. **RESULTS:** Ninety-four patients were included for analysis. No significant correlations could be demonstrated between OH and components of ABP (reversal of circadian pattern, postprandial hypotension and heart rate variability), nor between OH and autonomic reflex screen. Reversal of circadian pattern did not demonstrate significant correlation (r= 0.12, p=0.237) with autonomic reflex screen, but postprandial hypotension (r=0.39, p=0.003) and heart rate variability (r=0.27, p=0.009) demonstrated significant correlations. Postprandial hypotension was associated with a five-fold (OR 4.83, CI95% 1.6 - 14.4, p=0.005) increased risk and heart rate variability with a four-fold (OR 3.75, CI95% 1.3 - 10.6, p=0.013) increased risk for autonomic dysfunction. Per ROC curves, heart rate variability (0.671, CI95% 0.53-0.81, p=0.025) and postprandial hypotension (0.668, CI95% 0.52-0.72, p=0.027) were among the best predictors of autonomic dysfunction in routine clinical practice. **CONCLUSIONS:** Postprandial hypotension and heart rate variability on ambulatory blood pressure monitoring are strong predictors of autonomic dysfunction in routine clinical practice.


Funding: No

Funding Component: P126

**PI3 Kinase Regulation is Independent of Changes in Associated Phosphatases in Adult**
Female Sheep with Fetal Betamethasone Exposure

Alexa Hendricks, Hossam A. Shaltout, Mark C. Chappell, Debra I Diz, Wake Forest Sch of Med, Winston Salem, NC

Fetal exposure to betamethasone (BMX) when given to a woman threatening premature delivery may lead to negative long term consequences for autonomic regulation. In sheep, BMX in utero results in elevated mean arterial pressure (MAP), decreased baroreflex sensitivity (BRS) for control of heart rate and insulin resistance in adult offspring. This is accompanied by dysregulation of the brain, renal and circulating renin-angiotensin system (RAS) reflecting reduced brain medullary levels of angiotensin-(1-7) [Ang-(1-7)] and loss of the beneficial actions of this peptide within the nucleus of the solitary tract (NTS) for BRS regulation. Reports link acute NTS injections of Ang-(1-7) to PI3 Kinase (PI3K) activation resulting in an improved BRS in normal rats. The MAPK and PI3K pathways are implicated in regulation of both metabolic function and blood pressure. However, whether specific signaling pathways are altered chronically in association with lower Ang-(1-7) in brain medulla of fetal-programmed sheep is not known. Using a protein array and Western blot hybridization analyses, cytosolic homogenates of brain dorsomedial medulla from female BMX sheep (n = 8 sheep; 4 per group) exhibited lower expression of phosphorylated proteins in the PI3 kinase pathway (phosphorylated GSK3β -33 ± 0.10%, phosphorylated Akt -22 ± 0.33%; p < 0.05), whereas the MAPK proteins such as phosphorylated ERK and p38 were not altered. To investigate whether the lower phosphorylation pattern within the PI3K pathway results from disruption of regulatory phosphatases, we used Western blot hybridization and ELISA for three PI3K phosphatases: PTEN, PP2A and PTP1B. Relative expression of each were not statistically different in BMX females versus vehicle (ELISA concentration of PTP1B: Control 2.92 ± 0.30 vs BMX: 2.77 ± 0.59; PP2A to βactin, Control: 1.61 ± 0.62 vs BMX: 1.94 ± 1.47; PTEN to βactin, Control:1.53 ± 0.96 vs BMX:1.81 ± 1.17). We conclude that in utero BMX promotes an imbalance in adult brain medullary RAS and PI3K signaling pathways known to influence cardiometabolic regulation. This altered kinase pattern does not appear to be a result of changes in PI3K regulatory phosphatases, although direct measures of kinase and phosphatase activity are required to support this conclusion.


Funding: No

Funding Component: P127

EET-mediated Recruitment of PGC-1α, Restores Mitochondrial Function, LV Function, and Ameliorates Development of Cardiovascular Disease in Db Mice That is Reversed by Lentiviral- PGC-1α (Sh)

Lars Bellner, New York Medical Coll, Valhalla, NY; Jian Cao, Chinese PLA General Hosp, Beijing, China; Gregory Joseph, Joseph Schragenheim, Shailendra P Singh, New York Medical Coll, Valhalla, NY; Attallah Kappas, Rockefeller Univ, New York, NY; Nader Abraham, New York Medical Coll, Valhalla, NY

Background: Obesity and diabetes are independently associated with the development of cardiac events that occur in db mice that are linked to a decrease in heme oxygenase-1 (HO-1) and epoxyeicosatrienoic acids (EETs). An increase in HO-1 and EET levels is associated with a decrease of ROS, adiposity and increased mitochondrial function in several
animal models. The roles and inter-relationship of HO-1, EET, and PGC-1α in cardiomyopathy pathogenesis has not been investigated.

**Methods:** Obese (db) mice 5 wks of age were allowed to acclimate for 17 wks, and then divided into 3 groups. A) Control, B) EET-A in drinking water, and C) EET-A and lentiviral (Ln) PGC-1α Sh, which decreased PGC1α by 40-60 %. Echocardiography was performed at 5 and 34 wks, serum and heart tissue were harvested to measure signaling and mitochondrial fusion proteins. **Results:** Group A) developed hyperglycemia, insulin resistance, and LV dysfunction. These changes were associated with decreases in PGC-1α, HO-1, MnSOD, and mitochondrial fusion associated proteins; Mfn 1/2 and OPA-1: Group B), received EET-A, and displayed normal levels of glucose, adipose adiponectin (p<0.05), insulin sensitivity, and increased levels of HO-1, PGC1α (p<0.02), insulin receptor phosphorylation (p<0.05), and Mfn 1/2 and OPA1 (p<0.001) when compared to normal levels of young (5 wks) db mice, before the development of cardiomyopathy: and group C) inhibition of PGC1α by Ln-PGC1α (Sh) prevented EET from restoring LV function and fractional shortening (p<0.05) EET-A- Ln-PGC1α (Sh) animals display a worsening of intrinsic myocardial contraction compared to db control. This is likely related to a decrease in the cardiac mitochondrial network. The latter was reciprocally correlated to HO-1-PGC1α levels that diminished as obesity/diabetes progressed. **Conclusion:** An EET-A agonist ameliorates adiposity, BP elevation, mitochondrial function and cardiomyopathy as a result of increased levels of PGC1α and HO-1-expression. Suppression of PGC1α by Ln PGC1α (Sh) worsened insulin resistance suggesting targeting mitochondrial fusion/fission may be a promising therapy for diabetes and heart disease.


**Funding:** No

**Funding Component:** P128

**Enhanced NOS1β in the Macula Densa Contributes to the Diabetic Hyperfiltration**

**Jin Wei, Jie Zhang, Gensheng Zhang, Lei Wang, Shaohui Wang, Ruisheng Liu, Univ of South Florida, Tampa, FL; Jacentha Buggs, Tampa General Hosp, Tampa, FL**

Hyperfiltration is common in early diabetes and considered a risk factor for diabetic nephropathy. Inhibited tubuloglomerular feedback (TGF) mediated by less NaCl delivery at the macula densa contributes to the diabetic hyperfiltration. Nitric oxide (NO) released from macula densa via neuronal nitric oxide synthase (NOS1) inhibits TGF response. We recently demonstrated the significance of TGF response in the long-term control of GFR, sodium excretion and blood pressure, mediated by macula densa NOS1β. However, whether the NOS1β-mediated TGF response play an important role in the diabetic hyperfiltration is unknown. We hypothesized that macula densa NOS1β is upregulated in diabetes, which blunts TGF response and contributes to the diabetic hyperfiltration. The mice with deletion of NOS1 specifically from the macula densa (MD-NOS1KO) and wild-type C57BL/6 (WT) mice were used. Diabetes was induced by alloxan (55 mg/kg i.v.) with blood glucose from 350 to 450 mg/dl. Expression of NOS1 splice variants was measured with real-time PCR and Western blot. GFR was measured by plasma FITC-inulin clearance following a single bolus intravenous injection in conscious mice. GFR increased by 24.7% in diabetic WT mice (from 241.60±19.73 to 301.35±21.76 μl/min) and only by 16.5% in diabetic MD-NOS1KO mice (from 236.61±16.12
to 275.61±11.73 µl/min) (n=6/group, p<0.01 vs baseline; p<0.05 vs WT). To determine whether glucose induces hyperfiltration mediated by macula densa NOS1, intravenous infusion of 2 µl/g glucose solution (2 M) into non-diabetic C57BL/6 mice elevated blood glucose to about 350 mg/dl and increased GFR by 19.1% (from 236±15.4 to 281±9.7 µl/min, n=6, p<0.05), but did not significantly increase GFR (from 223±6.9 to 240±15.7 µl/min) in non-diabetic MD-NOS1KO mice (n=6, p<0.01 vs WT). The expression of NOS1β was upregulated by 8.9±1.3 folds in protein level and 10.1±2.1 folds in mRNA level in diabetic WT mice (n=4, p<0.01). Present study provided a novel mechanism for diabetic hyperfiltration mediated by macula densa NOS1β. Enhanced NOS1β in the macula densa contributes to the diabetic hyperfiltration.


Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P129

The High Blood Pressure-Malaria Protection Hypothesis

Julio Gallego-Delgado, NYU SoM, New York, NY; Thomas Walther, Sch of Med Univ Coll Cork, Cork, Ireland; Ana Rodriguez, NYU SoM, New York, NY

For decades, researchers have been fascinated by the idea of a causative connection between hypertension and malaria as the prevalence of hypertension is higher in populations that have been exposed to malaria for long periods. Cerebral malaria is a multi-factorial syndrome involving the interaction between P. falciparum-infected red blood cells (Pf-iRBC) and host cerebral microvascular endothelial cells. Disruption of the blood-brain-barrier (BBB), ranging from increased permeability to complete loss of inter-endothelial junctions (IEJ) and to petechial hemorrhages in the brain, is a characteristic feature of cerebral malaria. Our in vitro experiments show that Pf-iRBC induce the disruption of IEJ in human brain microvascular endothelial cells (HBMEC) mediated by the activation of β-catenin. Protection from this effect is achieved by blockade of the angiotensin II (Ang II) type 1 receptor (AT1) or stimulation of the type 2 receptor (AT2), which abrogate Pf-iRBC-induced activation of β-catenin and prevent the disruption HBMEC monolayers. Using a mouse model of cerebral malaria, we observed similar effects after treatment with Ang II receptors modulators, leading to protection against cerebral malaria, reduced cerebral hemorrhages (1.98 and 1.17 vs 0.63 for control, AT1 blocker and AT2 agonist respectively; p<0.05) and increased survival. While only 25% survived in controls (4 of 16 mice), 81.7% (13 of 15) and 71.4% (5 of 7) survived under AT1 blockade or AT2 agonist, respectively. In contrast, AT2-deficient mice were more susceptible to cerebral malaria (0% survival; 0 of 9). A causal association between high levels of Ang II and protection from malaria pathogenesis can provide a likely explanation for the increased prevalence in hypertension observed in populations of African and South Asian origin. Furthermore, this potential causative connection might also direct to unique approaches for the effective treatment of cerebral malaria.

J. Gallego-Delgado: None. T. Walther: None. A. Rodriguez: None.

Funding: No
Funding Component: P130
Association Between PFOA, PFOS and Obesity Among Children in a Nationally Representative Sample

Sarah D Geiger, Ping Yao, Elizabeth Rogers, Northern Illinois Univ, DeKalb, IL; Michael Vaughn, Zhengmin Qian, St. Louis Univ, St. Louis, MO

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are two types of perfluoroalkyl substances (PFASs) commonly used in the manufacturing process of many consumer products. Both have been detected in the blood of the majority of Americans. PFASs have been shown to be associated with intermediate cardiovascular disease (CVD) outcomes such as hypertension, hyperuricemia and dyslipidemia, but their relationship with obesity, a risk factor for intermediate and advanced CVD, remains largely unexplored. In this context, we examined the associations between PFOA and PFOS levels, and Body Mass Index (BMI) and waist circumference (WC) in a representative sample (N = 5,180) of US children. Our cross-sectional sample included participants aged 12-19 years from CDC’s National Health and Nutrition Examination Survey 1999-2000, 2003-2012. PFOA and PFOS were measured in ng/mL and modeled as quartiles of exposure, where quartile 1 is the referent group across models. Overweight/obesity was defined as age-, sex-specific BMI ≥85th percentile; abdominal obesity was defined as age-, sex-specific waist circumference ≥90th percentile. A multivariable model adjusting for age (years), sex (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), and annual household income category (<$25,000, $25,500-$54,999, $55,000 and over), revealed an inverse association between PFOS and overweight/obesity. Odds ratios (ORs) for overweight/obesity were 0.81 (95% Confidence Interval [CI] 0.14-0.58) for exposure quartile 2, 0.28 (0.11-0.58) for quartile 3, and 0.26 (0.15-1.05) for quartile 4 (p-trend=0.001). Results were similar for abdominal obesity where, for example, children in quartile 2 of PFOS exposure experienced a multivariable-adjusted OR of 0.42 (0.25-0.72; p-trend=0.023). PFOA was not found to be significantly associated with either outcome. Results are paradoxical in that PFASs may be protective against a risk factor for conditions with which PFASs are positively associated. Because we could not identify any temporal relationships between exposure and outcomes in this cross-sectional study, more studies with improved study design (such as a cohort study) are warranted to confirm the association.


Funding: No
Funding Component: P132

Role of NO/NOS System in Blood Pressure Regulation in Postmenopausal Female SHR with Hormone Replacement Therapy

Carolina Dalmasso, Rodrigo O. Maranon, Chetan N. Patil, Licy L. Yanes-Cardozo, Jane F. Reckelhoff, Univ of Mississippi Medical Ctr, Jackson, MS

Estradiol (E2) and testosterone (T) stimulate synthesis of nitric oxide (NO). Since the WHI study, postmenopausal women are given E2 and/or T supplements to alleviate the symptoms of menopause. NO is a key regulator of blood pressure (BP), mediating endothelial and vascular function. Studies from our lab showed that E2 plays little role in BP control in young female SHR; however, whether E2 replacement with or without T would reduce BP in postmenopausal female SHR is not known. If E2 does not reduce BP in old female SHR, it is
possible that there is a defect in the NO system that prevents the vasodilatory E2 effect mediated by NO. The hypothesis tested in this study was that E2/T replacement would reduce BP in old female SHR, and if not, the mechanism responsible is a deficient NO system that is incapable of upregulating in response to E2.

After baseline (B) mean arterial pressure (MAP; telemetry), female SHR (19 mos, n=5) were implanted with 17-β E2 (0.1 mg/pellet) and T (5mm in silastic tubes) and MAP was measured. After a transient reduction in MAP over 2-3 days (B: 175±5; E2+T: 161±4 mm Hg, p<0.05), MAP returned to baseline levels by day 4 (176±5 mm Hg). These data suggest that the acute vasodilatory response to E2/T in old female SHR was intact. On day 8 T tubes were removed, and MAP was measured for additional 16 days. Removal of T had no effect on BP (175±5 mm Hg). To evaluate NO system, rats were given: 1) 2% L-Arginine (L-Arg, 21 d); 2) 0.1% sodium nitrite (NaNO2, 6 d); 3) nitro-L-arginine methyl ester (L-NAME, 4mg/kg/d, 5 d). L-Arg supplement failed to change MAP (B: 175±5, L-Arg: 176±5 mm Hg, p<NS). In contrast, NaNO2 did decrease MAP (B: 176±5, NaNO2: 161±3 mm Hg, p<0.05), suggesting a deficient endogenous synthesis of NO but the ability of the old female SHR to respond to an NO donor. L-NAME increased MAP (B: 176±5; L-NAME: 189±3 mm Hg, p<0.05). In total, these data suggest that the NO system in old female SHR is capable of responding appropriately to NO donors or complete blockade. However, the lack of response to L-Arg suggests a deficiency in the ability to normally synthesize NO, and thus may in part be responsible for the lack of a depressor response to E2, and therefore, may contribute to the elevated BP in old female SHR. Supported by NIH-R01HL66072, PO1HL51971 (JFR), 14POST18640015 (ROM).


Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P133

Melanocortin 4 Receptor is Required for the Effect of Testosterone Supplement on Metabolic Parameters and Blood Pressure

Rodrigo O Maranon, Carolina Dalmasso, Frank T Spradley, Joey P Granger, Luis A Juncos, Jane F Reckelhoff, Univ of Mississippi Medical Ctr, Jackson, MS

Melanocortin 4 receptor (MC4R) activation causes appetite suppression and sympathetic nervous system activation. Blockade of MC4R increases food intake and in some cases, decreases BP. MC4R has been implicated as playing a role in obesity and hypertension. Previously we observed that testosterone supplementation in male Zucker rats improved insulin resistance and the characteristics of metabolic syndrome, but increased BP. Similarly, testosterone increases BP in male SHR, and blockade of the MC4R reduces BP in both young and old male SHR. In the present study we tested the hypothesis that testosterone supplementation (T) requires activated MC4R to attenuate metabolic parameters, and to increase BP. Male obese MC4R−/− and Wistar Hannover rats (WT) were treated with T (MC4R-KO+T vs WT+T: 22 mg/10 mm silastic pellet) or placebo (empty pellets; MC4R-KO+P, and WT+P), beginning at 10 wks of age for 6 weeks. In WT animals, T reduced body weight by 13% compared to placebo (367 gr vs 432gr; p<0.05), but had no effect on food intake (WT+P: 19±7 gr/d vs WT+T: 16±5 gr/d, n=3, p:NS). In MC4R-KO rats, body weight and food intake were similar in both placebo and T rats (BW: 555±20 gr vs 526±18 gr, and FI: 26±1 gr vs 28±2; n=3, p:NS, respectively). T reduced fat
mass (measured by ECHO-MRI®) in WT+T by 38% compared to placebo (50±2.9 gr vs 31±3.2 gr; p<0.05); however, there was no effect on fat mass in MC4R-KO + T (149±8 gr vs 147±11 gr; n=3, p:NS). Lean mass (by ECHO-MRI®) was not affected by T in either MC4R-KO+T or WT+T (p:NS). Finally, unlike our previous studies in obese Zucker and SHR males, T failed to affect mean arterial pressure or heart rate (by telemetry) in MC4R-KO+T rats compared to control MC4R-KO+P (MAP: 122±2 mmHg vs 122±1 mmHg; n=3, p:NS; HR:309±5 bpm vs 320±17 bpm; n=3, p:NS). These data suggest that the effect of T on metabolic parameters and BP may be mediated at least in part by MC4R since in the absence of active MC4R, T is not able to affect either body composition or BP. Also, these data gave a potential new insight into the mechanism by which T contributes to BP control and adipose tissue regulation. More studies are necessary to clarify the role of MC4R in mediating the effects of testosterone. Supported by NIH-R01HL66072, R01HL69194, PO1HL51971 (JFR), 14POST18640015 (ROM).


Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P134

Hypertension in Postmenopausal Women: Role of Renin-angiotensin System and Eicosanoids

Chetan N. Patil, Carolina Dalmasso, Rodrigo O Maranon, Licy L. Yanes-Cardozo, Jane F. Reckelhoff, Univ of Mississippi Medical Ctr, Jackson, MS

Hyperandrogenemic postmenopausal women have elevated blood pressure (BP) and the mechanisms responsible for it have not been elucidated. We tested the hypothesis that activated renin-angiotensin system (RAS) in combination with cytochrome P450 (CYP 450) arachidonic acid metabolites in renal microvasculature contribute to the elevated BP in post-estrous cycling hyperandrogenemic female (PMHAF) rats. Female SD rats were implanted with DHT (7.5mg/90d) or placebo pellets (n=12/group) beginning at 6 wks of age; pellets were changed every 85 d. At 14 months of age, baseline mean arterial pressure (MAP, telemetry) was measured in control and PMHAF rats. Each group was then divided into two groups, one group received non-specific CYP 450 inhibitor, 1-aminobenzotriazole (1-ABT; 100mg/kg/day, i.p.), and the other received Losartan (40mg/kg/day, P.O.) for 10 days. For the following 10 days all rats received both drugs. Baseline BP was significantly higher in PMHAF than controls (112±3 vs 126±3 mmHg, p=0.008). 1-ABT alone had no effect on BP in either control or PMHAF rats. Losartan alone reduced MAP in both groups (112±3 vs 100±3 mmHg, p<0.05 in control and 126±3 vs 100±3 mmHg, p<0.05 in PMHAF). In control rats first given 1-ABT, losartan had no effect on BP (112±3 vs 113±2 mmHg, p=NS). In control rats first given losartan, BP continued to decrease over the next 10 days (97±3 vs 87±2 mmHg, p=0.046). Just as in control rats, in PMHAF rats given 1-ABT first, losartan had no further effect on BP (130±2 vs 126±4 mmHg, P=NS). Unlike in control rats, PMHAF rats given 1-ABT after losartan, the BP increased to baseline levels (126±3 vs 116±5 mmHg, p=NS). This data suggests that BP in aging female control rats is mediated by the RAS and likely greater vasoconstrictor to vasodilator ratios of eicosanoids. BP control in PMHAF rats is more complex with the RAS playing a major role. Data suggests that vasodilators (EETs) in the renal microvasculature and 20-HETE in the tubules
contribute to BP control which would explain the increase in BP back to baseline with 1-ABT + losartan. Future studies will be necessary to determine exact mechanisms responsible for the hypertension in PMHAF rats, and may explain why in hyperandrogenemic women BP is difficult to control to normotensive levels.


Funding: No
Funding Component: P135

2-methoxyestradiol Minimizes Angiotensin II-induced Hypertension and Renal Dysfunction in Ovariectomized Female and Intact Male Mice

Ajeeth K Pingili, Shyamala Thirunavukkarasu, Nayaab S Khan, The Univ of Tennessee Health Science Ctr, Memphis, TN; Akemi Katsurada, Dewan S Majid, Gabriel L Navar, Tulane Univ, Sch of Med, New Orleans, LA; Kafait U Malik, The Univ of Tennessee Health Science Ctr, Memphis, TN

Men and post-menopausal females are more prone to develop hypertension and renal dysfunction as compared to pre-menopausal females. It is well documented that in various experimental models of hypertension, the protection against hypertension in females is lost following ovariectomy (OVX). Recently we have shown that CYP1B1 protects against angiotensin II (Ang II)-induced hypertension and associated cardiovascular changes in female mice, most likely via production of 2-methoxyestradiol (2-ME). This study was conducted to determine if 2-ME reduces Ang II-induced hypertension, renal dysfunction and end organ damage in OVX female, and intact male mice. Treatment of OVX Cyp1b1−/+ and Cyp1b1−/− female mice with 2-ME (1.5 mg/kg/day i.p., for 2 weeks) reduced Ang II-induced increase in systolic blood pressure (SBP) (182±5.1 vs. 143± 2.4 mmHg, 179±6.4 vs. 140± 8.6 mmHg, P < 0.05, n= 5), water consumption, urine output and osmolality, and proteinuria (5.5±0.7 vs. 3.3±0.5 mg/24 hrs, 8.4±1.3 vs. 4.4 ±0.9 mg/24 hrs) respectively. 2-ME also reduced Ang II-induced increase in SBP (188±2.6 vs. 143± 2.7 mmHg, P < 0.05, n= 5) in intact male mice. 2-ME did not alter water consumption and urine osmolality, but reduced urine output and sodium excretion, and proteinuria (14.4±2.0 vs. 6.0±0.5 mg/24 hrs) in intact Cyp1b1−/+ male mice. Treatment with 2-ME attenuated Ang II-induced end-organ damage (actin and collagen accumulation) in OVX Cyp1b1−/+ and Cyp1b1−/− female and Cyp1b1−/+ male mice. 2-ME mitigated urinary excretion of angiotensinogen in OVX Cyp1b1−/+ and Cyp1b1−/− female mice infused with Ang II. These data suggest that 2-ME reduces Ang II- induced hypertension and associated renal dysfunction and end-organ damage in OVX Cyp1b1−/+ and Cyp1b1−/− female, and intact male mice. Therefore, 2-ME could serve as a therapeutic agent for treatment of hypertension and associated pathogenesis in post-menopausal females, and intact males.


Funding: No
Funding Component: P136

Dietary Salt and Non-Salt Components Have Substantial Effects on Genome-Wide DNA Methylation Patterns in Dahl SS Rats

Justine M Abais-Battad, Pengyuan Liu, David L Mattson, Yong Liu, Allen W Cowley Jr, Theresa M Kurth, Chun Yang, Hayley Lund, Aron M Geurts, Srividya Kidambi, Theodore A Kotchen,
Epigenetic modifications of the genome play a key role in the regulation of gene expression. It has been reported that epigenetic modifications of several genes are associated with hypertension. To investigate the potential role of genome-wide changes in DNA methylation in salt-induced hypertension, experiments were performed on inbred Dahl SS rats obtained from two colonies maintained at the Medical College of Wisconsin (i.e. MCWSS) and Charles River Laboratory (CRLSS). The colonies are genetically identical, but CRLSS rats were maintained on a whole grain diet containing 1% NaCl (CRLSS_LS) while MCWSS rats were fed casein-based AIN-76A chow containing 0.4% NaCl (MCWSS_LS) until both colonies were switched to an AIN-76A chow containing 4% NaCl for 14 days starting at 6 weeks of age (CRLSS_HS and MCWSS_HS, respectively). Mean arterial pressure and albumin excretion rate in MCWSS_HS rats were significantly greater (142±14 mmHg and 100±16 mg/day, n=6) than in CRLSS_HS rats (118±2 mmHg and 20±2 mg/day, n=7). Reduced representation bisulfite genome sequencing (RRBS) measured 5-Methylcytosine levels at single-base resolution in the renal outer medulla in the above groups, each with four biological replicates. For genomic regions located within CpG islands (CGI’s) and exhibiting differential methylation between LS and HS in each colony, HS diet increased median methylation levels several-fold in both MCWSS (7.45% vs. 0.35% for MCWSS_HS vs. MCWSS_LS, respectively, p = 2.84E-31) and CRLSS rats (7.62% vs. 1.21% for CRLSS_HS vs. CRLSS_LS, respectively, p = 1.65E-32). For genomic regions exhibiting differential methylation between MCWSS and CRLSS, MCWSS_HS rats (which exhibited higher blood pressure) had higher median methylation levels than CRLSS_HS rats (7.56% vs. 2.75%, p = 2.12E-9). We observed 156 hypermethylated and 241 hypomethylated regions within CGI’s of MCWSS_LS compared to CRLSS_LS. Examples of differentially methylated genes include the serine protease Prss2, the transcription factor E2f1, and the matrix protein Spock2. These results suggest that sodium-dependent and independent dietary components could induce changes in DNA methylation that may predispose and participate in the development of hypertension and renal damage.

J.M. Abais-Battad: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. P. Liu: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. D.L. Mattson: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. Y. Liu: None. A.W. Cowley: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. T.M. Kurth: None. C. Yang: None. H. Lund: None. A.M. Geurts: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. S. Kidambi: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. T.A. Kotchen: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. M. Liang: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. NIH.

Funding: Yes
Mir-122 is Associated With Stress-response Protein, Hemeoxygenase-1, for Regulation of Extracellular Matrix Remodeling in Renal Hypertension

Biswa P Das Purkayastha, Lu Ren, Sathnur Pushpakumar, Utpal Sen, Univ of Louisville, Louisville, KY

Oxidative stress is a major contributing factor in hypertension-induced kidney injury. Hemeoxygenase-1 (Ho-1) is stress response protein constitutively expressed by the proximal tubular epithelial cells in response to oxidative stress. MicroRNAs are single stranded RNA involved in the regulation of gene expression. MicroRNA-122 has been shown to regulate Ho-1 expression in hepatitis; however whether miR-122 regulates Ho-1 in hypertensive kidney is not known. The purpose of the study was to investigate the miRNA-122 Ho-1 regulation and determine its role in extracellular matrix remodeling in renal hypertension.

In vitro experiments were done using mesangial cells, treated with/without 200 μM of Angiotensin-II (Ang-II). Ho-1 was induced by ~3.5 folds with Ang-II treatment. miR-122, Ho-1 regulator, was downregulated by >15 times in Ang-II treated cells.

In vivo experiments were performed on WT (C57BL6/J) mice aged 12-14 wk and 75-78 wk. The animals were treated with Ang-II (1000ng/kg/min) for 4 weeks. Ho-1 is ~6 folds less in kidney of aged mice as compared to that in the young mice. Hypertension increases miR-122 expression to a greater extent (~5 folds) in aged animals. In Ho-1 knocked down mesangial cells, the extracellular matrix component, Collagen 1A1 (Col1a1), was increased by ~2 folds. In contrast, vascular endothelial growth factor (Vegf) and hypoxia-inducible factor (Hif1α) were downregulated in Ho-1 depleted cells. In conclusion, micro RNA, miR-122, transcriptionally regulates Ho-1 as a repressor in kidney and thus affects Ho-1 mediated regulation of the extracellular remodeling in hypertension-induced renal damage.

B.P. Das Purkayastha: None. L. Ren: None. S. Pushpakumar: None. U. Sen: None.

Funding: No

Targeted Disruption of Regulated Endocrine Specific Protein (Resp18) in Dahl SS Rats Increases Blood Pressure and Renal Injury

Sivarajan Kumarasamy, Harshal Waghulde, Ctr for Hypertension and Personalized Med, Dept of Physiology and Pharmacology, Univ of Toledo Coll of Med and life Sciences, Toledo, OH; Steven Haller, Ctr for Hypertension and Personalized Med, Dept of Med, Univ of Toledo Coll of Med and life Sciences, Toledo, OH; Blair Mell, Xi Cheng, Bina Joe, Ctr for Hypertension and Personalized Med, Dept of Physiology and Pharmacology, Univ of Toledo Coll of Med and life Sciences, Toledo, OH

Essential hypertension is a complex polygenic trait. To understand the genetics of Blood pressure (BP) control, loci are mapped using populations derived by crossing several rat strains with the genetically hypertensive rat model, the Dahl SS rat (SS) and validated as BP quantitative trait loci (QTL). Genes located within the mapped BP QTLs are candidates as inherited loci controlling BP, but require further proof. One such mapped locus is on rat chromosome 9, wherein the proof for one of the candidate genes Regulated Endocrine Specific Protein-18 (Resp18), as a BP QTL, is currently inadequate. To ascertain the status of Resp18 as a BP QTL, a custom targeted gene disruption model of Resp18 was developed on the SS background. As a result of this ZFN
mediated disruption, a 7 bp deletion occurred within exon 3 of the Resp18 locus, resulting in a truncated protein with 111aa compared to the full length protein consisting of 175aa. Under a high salt dietary regimen, both systolic and diastolic BP of Resp18<sup>mutant</sup> rats were significantly increased compared to SS rats (151±3 vs. 170±6mmHg; 116±2vs. 129±4mmHg n=10-12, p<0.05). Resp18<sup>mutant</sup> rats demonstrated higher proteinuria compared to SS rats (221±14 vs. 268±14mg of protein/kg body weight/24hr; n=14-25, p<0.05). In vascular reactivity experiment, Resp18<sup>mutant</sup> rat mesenteric arteries demonstrated significantly reduced relaxation as compared to SS rats (n=4, p<0.05). An associated decrease in sodium excretion and an increase in glucose excretion were also observed in urine samples of Resp18<sup>mutant</sup> rats compared to SS rats (51±7.3vs.27±2.7meq/L/24hr; 10±0.3 vs. 14±1.4mg/dl/24hr, n=5-8, p<0.05). Renal histology examination revealed that Resp18<sup>mutant</sup> rat kidneys showed increased fibrosis compared to SS rats. The median survival of Resp18<sup>mutant</sup> rats was 259 days, which was significantly lower than the median survival of 309 days for the SS (n=8-16, p<0.05). In conclusion, the data suggest that Resp18 is a gene associated with the development of hypertension, renal disease and increased mortality in the SS rats. Resp18 is a molecule involved in the secretory pathway and thereby, future studies will be conducted to examine the mechanistic links between Resp18, its function in the secretory pathway and BP regulation.


Funding: No

Funding Component: P139

Yingchuan Li, Ctr of Systems Molecular Med, Dept of Physiology, Medical Coll of Wisconsin, Milwaukee, WI; Srividya Kidambi, Dept of Med, Medical Coll of Wisconsin, Milwaukee, WI; Pengyuan Liu, Michelle L Leitlt, Yong Liu, David L Mattson, Allen W Cowley, Jr, Ctr of Systems Molecular Med, Dept of Physiology, Medical Coll of Wisconsin, Milwaukee, WI; Theodore A Kotchen, Dept of Med, Medical Coll of Wisconsin, Milwaukee, WI; Mingyu Liang, Ctr of Systems Molecular Med, Dept of Physiology, Medical Coll of Wisconsin, Milwaukee, WI

Introductions: Historic DNA samples represent a potentially highly valuable resource for epigenetic analysis. Reduced representation bisulfite sequencing (RRBS) is a cost-effective, near genome-wide method for quantifying DNA methylation levels at single base resolution. It is unknown whether historic DNA samples stored for a long time (15-20 years) under various conditions could maintain stable methylation profiles as determined by RRBS. Methods: We used 5 groups of DNA samples (n=4 in each group) to compare and evaluate the stability of DNA methylation profiles under standard storage conditions of 4°C since 1996. Group 1 had been extracted from EDTA-anticoagulated blood and stored at 4°C since 1996; Group 2 had been diluted from group 1 to 10ng/µl and stored at -20°C since 2009; Group 3 were extracted from citrate-anticoagulated blood stored at -80°C since 1996; Group 4 were extracted from fresh EDTA-anticoagulated blood; Group 5 were extracted from fresh citrate-anticoagulated blood. The samples in groups 1, 2 and 3 were from the same subjects. All DNA samples were processed for RRBS libraries and then sequenced. Results: The global methylation level of group 1 was lower than group 2 (36.88±4.12% vs 43.66±1.29%, p=0.04), but was not statistically different from group 3 (44.31±1.46%, p=0.07). The methylation level of groups 4 and 5...
(40.34±3.73% and 38.64±1.94%) was not significantly different. In addition, group 4 and 5 were not significantly different from group 1. The correlation of methylation levels for all CpG sites between individual samples in group 1 (0.987±0.001) was higher than that in group 2 and group 3 (0.984±0.002, p=0.01, and 0.969±0.015, p=0.01) and not significantly different from group 4 or 5. For CpG sites within CpG islands (CGI), the correlation between individual samples was not significantly different between any of the 5 groups. Moreover, the samples from the same subject show high correlation levels between groups 1, 2, and 3 for all CpG sites (0.987±0.006) and CpG sites within CGI (0.983±0.009). Conclusion: DNA samples stored at 4°C for prolonged periods of time are amenable to RRBS analysis. In addition, there were no significant differences in the DNA methylation profiles between citrate- and EDTA-anticoagulated blood.


Funding: Yes
Funding Component: National Center P140

Epigenetic Regulation of Nox4 in Hypertension-induced Injury in Aged Kidney

Lu Ren, Biswa Das Purkayastha, Utpal Sen, Sathnur Pushpakumar, Univ of Louisville, Louisville, KY

The prevalence of hypertension increases with age. At the cellular level, oxidative stress is a major contributing factor to the pathogenesis of hypertension-induced kidney damage. The nicotinamide adenine dinucleotide phosphate (NADPH) family is one of the major sources of reactive oxygen species generation in the body. Although the isoform, NADPH oxidase 4 (Nox4) is highly expressed in the kidney, its role in kidney diseases remains controversial as both pathogenic and protective effects have been described. In addition, its role in hypertension-induced kidney damage in aging remains unexplored. Current evidence supports the involvement of epigenetics in oxidative stress. The aim of the present study was to investigate the role of Nox4 in Ang-II induced kidney damage in aged mice and the potential role for epigenetic regulation. Wild type (WT, C57BL/6J) mice aged 12-14 wk and 75-78 wk were used in this study and treated with Ang-II (1000 ng/kg/min) for 4 weeks. Control mice received saline infusion. Blood pressure (BP) was measured twice weekly. Aged mice exhibited higher mean BP than young mice after Ang-II treatment (135.76±4.8 vs. 118.7±12.28 mmHg) and decreased renal vascular density on barium angiography. Dihydroethidium staining revealed increased oxidative stress in hypertensive kidney of aged mice. In the aged kidney, protein expression of mitochondrial antioxidant enzymes, manganese superoxide dismutase and catalase was decreased by > 2-fold compared to young, and decreased further after Ang-II infusion. The renal sensors of energy and redox state, SIRT1 and SIRT3, showed similar decrease in aged kidney compared to young kidney in response to Ang-II. Nox4 and p22phox protein expression was upregulated in the hypertensive kidneys of aged mice. The epigenetic mechanism involving DNA methylation showed increased DNA methyltransferases, DNMT1, 3a and 3b in the aged kidney. Following Ang-II, the aged kidney showed further increase of DNMT1 and 3a and a decrease in DNMT3b. Conclusion: Endogenous expression of Nox4 is upregulated in hypertensive kidney and is associated with alteration of DNA methyltransferases suggesting epigenetic regulation of oxidative stress in aged mice.
MicroRNAs regulate several physiological processes and are implicated in various pathologies, including hypertension. Previous work indicates miR-132 targets Sirtuin 1 (Sirt1), a histone deacetylase and regulator of epigenetic gene silencing in various cellular processes. Sirt1 is expressed in the kidney; however, its role in hypertensive kidney and whether it is regulated by physiological gaseous molecules, such as hydrogen sulfide (H₂S), is not known. In this study, we sought to determine the role of miR-132 in regulating Sirt1, Ace2 and At1 in hypertensive kidney and whether H₂S donor, GYY4137 (GYY), could reverse these effects and mitigates renal dysfunction. Wild-type mice were treated without or with Ang-II (1000 ng/Kg/Min) and GYY (133 µM) for 4 weeks. Quantitative PCR, Western blot, and immunofluorescence assays were performed. Increased expression levels of miR-132 in hypertensive mice (3.79 fold vs control) were reduced in mice receiving GYY treatment (2.43 fold vs control). Sirt1 expression was reduced (-1.15 fold) in Ang-II mice but was upregulated in GYY (1.25 fold) and Ang-II+GYY (1.9 fold) groups. A similar effect was seen with Sirt1 protein where the expression was increased in animals treated with GYY and Ang-II+GYY (1.16, 1.03 respectively) compared to Ang-II (0.47). Ace2 in Ang-II+GYY (0.45) was increased compared to Ang-II (0.17), while At1 was reduced (0.46) compared to Ang-II (0.86). Immunofluorescence showed decreased signal of Sirt1 in the glomerulus in Ang-II mice and increased At1 in the blood vessels surrounding the glomerulus, leading to constriction of renal artery, decreased blood flow, and kidney dysfunction. These effects were alleviated in mice treated with GYY. Our data suggests that upregulation of miR-132 in hypertensive kidney decreases Sirt1 and Ace2 expression, leading to increased Ang-II signaling through the At1 receptor and GYY supplementation reverses these expression patterns, leading to increased blood flow and kidney function.

Silas A Culver, Syed S. Quadri, Helmy M. Siragy, Univ of Virginia, Charlottesville, VA

Obesity represents a state of chronic inflammation in adipose tissue and this inflammation contributes to obesity related comorbidities. Our laboratory has previously demonstrated that the (Pro)renin receptor (PRR) promotes the production of proinflammatory factors via the ERK-MAP kinase pathway. We have also shown that in the setting of renal inflammation, NF-κB increases renal expression of PRR by binding to the PRR promoter. In this study we hypothesized that inflammation increases expression of PRR in adipose tissue. We monitored changes in expression of PRR mRNA by RT-PCR and protein by western blot in 3T3-L1 adipocytes treated with 100ng/ml LPS or normal culture medium (control) for 24 hours. To confirm that LPS treatment effectively induced an inflammatory
response in 3T3-L1 adipocytes, we monitored production of the inflammatory cytokine, IL-6, in culture media by luminex assay. Compared to control, LPS significantly increased IL-6 production (48.8 pg/mL in controls vs. 1877 pg/mL in LPS treated cells, p<0.05). Similarly, compared to control, LPS treatment increased adipocyte expression of PRR mRNA and protein by 120% and 62%, (p<0.05) respectively (figure 1). We conclude that inflammation increases adipocyte expression of PRR, suggesting that PRR may play a role in obesity related adipose tissue inflammation and obesity associated pathology.

S.A. Culver: None. S.S. Quadri: None. H.M. Siragy: None.

Funding: No
Funding Component: P143

Hypertension and Inflammation-Related microRNAs

Jamie G Hijnns, Tyler D Bammert, Philip J Kavlich, Kyle J Diehl, Grace M Linenberg, Ma’ayan V Levy, Jared J Greiner, Univ of Colorado Boulder, Boulder, CO; Brian L Stauffer, Univ of Colorado Sch of Med, Aurora, CO; Christopher A DeSouza, Univ of Colorado Boulder, Boulder, CO

microRNAs (miRs) are short single stranded noncoding RNAs that are involved in the regulation of a number of physiological and pathological processes. miRs down regulate target gene expression post-transcriptionally by degrading messenger RNA and/or by blocking translation. It is now recognized that miRs play a key role in regulating inflammation, vascular health and in-turn, cardiovascular disease (CVD). For example, altered expression of specific miRs such as, miR-126, miR-146a and miR-150 have been linked with heightened vascular inflammation and CVD risk.

Hypertension is associated with increased inflammatory burden. The mechanisms underlying blood pressure-related inflammatory stress are not fully understood. It is currently unknown whether inflammation-related miRs are dysregulated with elevated blood pressure. Accordingly, the aim of this ongoing study is to determine the influence of hypertension, independent of other risk factors, on circulating expression of miR-34a, miR-92a, miR -126, miR-146a and miR-150. To date, 28 sedentary, middle-aged adults have been studied: 14 normotensive (NT; 12M/2F; age: 53±1 yr; BP: 114/71±2/1 mmHg) and 14 hypertensive (HT; 12M/2F; 56±2 yr; 142/90±2/2 mmHg). All subjects were non-smokers, normolipidemic, non-medicated and free of overt CVD.

Circulating expression of miRs was determined in plasma using standard RT-PCR techniques with miR primers of interest. Expression was normalized to exogenous C. elegans miR-39 and reported as relative expression in arbitrary units (AU). Circulating expression of miR-126 (0.14±0.03 vs 0.33±0.04 AU) and miR-150 (0.06±0.02 vs 0.12±0.02 AU) were markedly lower (~135% and 100%, respectively; P<0.05) in the HT vs NT groups. There was no significant group difference in miR-34a (0.017±0.005 vs 0.010±0.001 AU), miR-92a (0.66±0.16 vs 1.0±0.13 AU) and miR-146a (0.04±0.01 vs 0.06±0.01 AU). Lower expression of miR-126 and miR-150 is consistent with a
proinflammatory phenotype, as both are involved in limiting inflammatory pathways. In summary, these initial results suggest that dysregulation of key inflammation-related miRs may contribute mechanistically to the heightened inflammatory state associated with elevated blood pressure and deserve further study.


Funding: No
Funding Component: P144

DHA is a Superior Treatment Over 12/15-lipoxygenase Inhibition in LPS-induced Acute Kidney Injury via Increased Resolvin D2 and IL-10 Levels

Mohamed Katary, Augusta university, Damnhour Univ, Augusta, GA; Nehal M Elsherbini, Augusta Univ, Mansoura Univ, Augusta, GA; Ahmed S Ibrahim, Augusta university, Mansoura Univ, Augusta, GA; Mohamed Al-Shabrawey, Augusta university, Augusta, GA; Ahmed A Elmarakby, Augusta university, Damnhour Univ, Augusta, GA

Acute kidney injury (AKI) is characterized by loss of kidney function and is often associated with high mortality rate. The polyunsaturated, omega-3 fatty acid, docosahexaenoic acid (DHA), has a promising role in preventing AKI; however; DHA reno-protective mechanism remains unclear. Our aim is to investigate whether and how DHA attenuates lipopolysaccharide (LPS)-induced AKI. Four groups of wild type C57BL/6 (WT) mice were used; control, LPS injected (4 mg/kg i.p), LPS treated with the 12/15-lipoxygenase (12/15-LO) inhibitor baicalein (20 mg/kg i.p for 6 days) and LPS treated with DHA (50 mg/kg i.p for 6 days). LPS-injected mice showed significant elevation in markers of renal injury as urinary excretion levels of protein, podocalyxin and albumin were elevated compared to WT untreated mice (albuminuria was 112±4 vs. 8±1 μg/day and podocalyxin was 3.5±0.6 vs. 0.6±0.1 μg/day in LPS injected mice vs. control WT, P< 0.05). The elevation in renal injury markers were also associated with increases in urinary excretion of thiobarbituric acid reactive substance (TBARs), as a marker of oxidative stress and monocyte chemoattractant protein-1 (MCP-1), as a marker of inflammation, in LPS injected mice. Treatment of LPS injected mice with either DHA or baicalein significantly reduced markers of renal injury, inflammation and oxidative stress. DHA or baicalein treatment also significantly reduced the elevation in renal mRNA expression levels of intercellular adhesion molecule 1 (ICAM-1) and tumor necrosis factor-α (TNF-α) in LPS injected mice. DHA was superior over baicalein in reducing renal expression of Interleukin-6 (IL-6) and IL-1ß and in increasing the renal expression of the anti-inflammatory cytokine IL-10 in LPS injected mice. DHA reduced renal tubular necrosis and vaculated cells and lowered renal expression of CD45, a marker of leucocyte adhesion, in LPS injected mice. DHA also restored the decrease in plasma resolvin D2 (RvD2) in LPS injected mice. In conclusion, our data suggest that 12/15-LO activation is involved in LPS-induced acute kidney injury and DHA is a superior treatment over 12/15-LO inhibitor in preventing LPS-induced kidney injury, at least in part, via 12/15-LO-induced resolvin D2.


Funding: No
Funding Component: P145
Renal Inflammation and Injury is Associated with Increased Lymphangiogenesis in Hypertension

Sterling C. Kneedler, Lauren Phillips, Kayla R. Hudson, Katharine M. Beckman, Texas A&M Health Science Ctr, College Station, TX; Alan R. Parrish, Univ of Missouri Sch of Med, Columbia, MO; Peter A. Doris, Univ of Texas Health Science Ctr at Houston, Houston, TX; Brett M. Mitchell, Texas A&M Health Science Ctr, College Station, TX

Hypertension is associated with immune system activation and inflammation. Renal infiltration of both innate and adaptive immune cells contributes to injury, dysfunction, and increased blood pressure. Activated immune cells that exit blood vessels into the interstitium then travel through lymphatic vessels to draining lymph nodes where they signal to other immune cells to increase the immune response. It is unknown how renal lymphatic vessels change in the context of hypertension, immune system activation, inflammation, and injury. We hypothesized that renal macrophage infiltration, inflammation, and injury would significantly increase lymphangiogenesis in various strains of rats. SHR rats that exhibit hypertension and renal injury (SHR-A3 strain) had significantly increased numbers of renal lymphatic vessels at 40 weeks of age compared to WKY controls (total of 3 fields of view: 52 ± 1 vs. 28 ± 1; p<0.05). This was associated with increased renal macrophage infiltration. SHR rats that exhibit hypertension but minimal renal injury (SHR-B2 strain) had significantly less renal lymphatic vessel numbers compared to WKY controls (25 ± 2 vs. 28 ± 1; p<0.05) and normal levels of macrophages. The signals for lymphangiogenesis, VEGF-C and its receptor VEGF-R3, were both increased significantly at the protein level in the kidneys of SHR-A3 rats at 18 weeks but not different in the kidneys of SHR-B2 rats compared to WKY controls. To test whether the increased lymphangiogenesis is due to hypertension and/or renal inflammation and injury, we obtained kidneys from Fischer 344 rats that exhibit normal blood pressure but develop renal inflammation and injury as they age. Compared to kidneys from control 4-month old Fischer rats, kidneys from 20-month and 24-month old Fischer rats had significantly increased numbers of lymphatic vessels (32 ± 3 vs. 74 ± 1 vs. 110 ± 6, respectively; p<0.05) and this was also associated with increased macrophage infiltration. Protein levels of VEGF-C and VEGF-R3 were increased significantly in 20-month old Fischer rats compared to 4-month old controls. These data together demonstrate that renal immune cell infiltration, inflammation, and injury increases lymphangiogenesis.


Funding: No

Funding Component: P146

Transient Neonatal High Oxygen Exposure Accelerates Adult Cardiac Baseline and Ang II-induced Prooxidative and Proinflammatory Responses

Muhammad Oneeb Rehman Mian, Fauve Boudreau, Mariane Bertagnolli, Marie-Amelie Lukaszewski, Fetomaternal and Neonatal Pathologies Axis, Res Ctr of Sainte-Justine Univ Hosp, Univ of Montreal, Montreal, QC, Canada; Thuy Mai LUU, Anne-Monique Nuyt, Fetomaternal and Neonatal Pathologies Axis, Res Ctr of Sainte-Justine Univ Hosp and Faculty of Med, Univ of Montreal, Montreal, QC, Canada
**Objective:** Preterm birth is associated with disequilibrated early life oxidant-antioxidant and proinflammatory conditions that can be carried into adulthood and contribute to the development of organ dysfunction. Neonatally high O2-exposed rats, a model of prematurity-related prooxidative conditions, exhibit adult hypertension, early cardiac dysfunction and fibrosis, and premature angiotensin (ANG) II-induced heart failure. We hypothesized that neonatal high O2 exposure will exaggerate baseline and ANG II-induced cardiac prooxidative and proinflammatory gene expression in adult rats.

**Design and Methods:** Sprague-Dawley pups were kept in 80% O2 (H group) or room air (NNI group) from day 3-10 of life. Twelve-weeks-old H and NNI rats were infused with ANG II (100 ng.kg⁻¹.min⁻¹) or saline (NaCl) for 4 weeks (n = 4 per group). RNA extracted from hearts was used in arrays to assess expression of oxidative stress and inflammatory genes. Data is expressed as fold change (F.C.) compared to control NNI+NaCl.

**Results:** H+NaCl exhibited increased baseline *Cd68* expression (F.C. 1.47; P<0.05 vs control), tendency of increased *Nfkb1* and *Tnf* expression (1.52 and 1.48), and a tendency of decreased *Il1b* and *Il18* expression (0.72 and 0.78).

H+ANGII versus NNI+ANGII exhibited similar increase in *Tnf* (2.12 vs 2.13; P<0.05 vs control), greater increase in *Cd14*, *Cd68*, and *Nfkb1* (2.5 vs 1.78, 2.13 vs 1.94, 2.0 vs 1.7; P<0.05 vs controls), and a greater tendency of increase in *Tgfβ1*, *Myd88*, *Cybb*, and *Gstp1* (2.68 vs 1.82, 2.37 vs 1.31, 1.91 vs 0.98, 2.45 vs 1.61) expression. NNI+ANGII versus H+ANGII exhibited greater increase in expression of genes involved in early inflammatory process, including *Ccl2*, *Icam1*, and *Vcam1* (2.07 vs 1.56, 1.88 vs 1.36, 2.34 vs 1.29; P<0.05 vs control).

**Conclusions:** Neonatal high O2 exposure accelerates baseline and ANG II-induced cardiac prooxidative and proinflammatory responses in adult rats, which could account for early cardiac abnormalities and premature ANG II-induced heart failure in this model.


Funding: No

Funding Component: P147

**Production of Anti-inflammatory Peptide N-Acetyl-Seryl-Aspartyl-Lysyl-Proline (Ac-SDKP) in Kidney**

Cesar A Romero, Nitin Kumar, Nour-Eddine Rhaleb, Oscar A Carretero, Henry Ford Hosp, Detroit, MI

Ac-SDKP is a natural peptide with anti-fibrotic and anti-inflammatory properties in vascular, myocardial and kidney tissues. It is released from thymosin B4 (TB4) by two step enzymatic reactions by meprin and the prolyl oligopeptidase enzymes (POP) and degraded by angiotensin converting enzyme (ACE). Treatment with ACE inhibitors (ACEi) increases Ac-SDKP plasma concentration. Kidney has been proposed as clearance organ for Ac-SDKP. Thus after Ac-SDKP filtration, 90% is degraded and only 10% is excreting in the urine. However, in rodents the decrease of glomerular filtration rate did not affect the amount of Ac-SDKP in urine and prevention of Ac-SDKP filtration at the glomerular level by using neutralizing antibodies also did no change the urinary Ac-SDKP content; This suggest that mechanisms other than only filtration can be present. We hypothesized that Ac-SDKP is produced in the kidney. We evaluated the presence of TB4 and POP mRNA by analyzing the trascriptome in each segment of the nephron using the public access NHLI database ESBL. We confirmed kidney expression of POP enzyme by immunohistochemistry (IHC). Finally the stop flow pressure technique was used to evaluate...
the Ac-SDKP formation in different segments of the nephron, in normal condition, under POP inhibition (POPi) and ACEi. All experiments were performed in Sprague-Dawley rats. TB4 mRNA was present in all the nephron segments, mainly in the distal parts (1941±949.8 RPKM) in comparison with proximal tubule (101.4±73.7 RPKM) (p<0.05). POP enzyme mRNA was present in proximal tubule, loop of Henle (inner medulla) and distal nephron (distal, connecting and collecting tubules). POP enzyme expression by IHC was observed in the distal convoluted tubule in cortex and mainly in the medullary region. The Stop flow technique showed the high Ac-SDKP/Inulin ratio in the distal nephron: 10.5±0.8 vs. 4.2±0.1 in the proximal segments (p<0.01). POpi infusion into the kidney decreased Ac-SDKP/Inulin in comparison to the vehicle group in distal (10.5±0.8 vs. 5.6±0.8, p<0.01) and proximal nephron segments (4.2±0.1 vs. 2.1±0.2, p<0.01). ACEi increased the Ac-SDKP content in all nephron segments, mainly in the distal part. We conclude that Ac-SDKP is synthetized in kidney, predominantly in the distal nephron.

C.A. Romero: None. N. Kumar: None. N. Rhaleb: None. O.A. Carretero: None.

Funding: No

Funding Component: P148

Connecting Tubule-glomerular Feedback (ctgf) in Renal Hemodynamics and Blood Pressure (bp) After Unilateral Nephrectomy (unx)

Cesar A Romero, Sumit Monu, Robert Knight, Oscar A Carretero, Henry Ford Hosp, Detroit, MI

Afferent arteriole resistance is regulated in part by myogenic response, tubuloglomerular feedback (TGF) and connecting tubule-glomerular feedback (CTGF). CTGF dilates afferent arteriole in response to high sodium in connecting tubule (CNT) counteracting and shifting to the right the TGF response (resetting); CTGF increases renal blood flow and glomerular pressure, both favoring glomerular filtration and sodium excretion. CTGF is initiated by epithelial sodium channel (ENaC) in CNT and inhibited by Benzamil. Unilateral nephrectomy (UNx) is accompanied by TGF resetting, increase in renal blood flow (RBF) and single nephron GFR in the remnant kidney, without any changes in systemic BP. We evaluated the effects of CTGF in TGF resetting, RBF and BP after UNx. UNx was performed on Sprague-Dawley rats and 24h later TGF was evaluated in vivo by renal micropuncture techniques by measure stop flow pressure (Psf). CTGF was evaluated by the differences between TGF maximal responses (TGFmax) with or without tubular benzamil perfusion. Another set of animals received chronic infusion of benzamil directly into the kidney, that started 1 week before UNx. Renal blood flow (RBF) was measured by arterial spin labeling-MRI 24h before and 24h after the UNx. Direct BP measurement was performed before and 3 weeks after the UNx. After UNx, TGF resetting was observed in UNx rats (TGFmax 8±1 vs. 1±1 mmHg, Sham vs. UNx; p<0.05). This TGF resetting was inhibited by benzamil. RBF increased after the UNx in comparison to sham and this increase was prevented by chronic infusion of benzamil into the kidney (Sham: 3±0.6; UNx: 4.6±0.3; UNx+Benzamil 3.5±0.6 ml/min/g tissue p<0.002). Basal mean BP values were not different between the vehicle or treated rats before the UNx; however 3 weeks after the UNx, rats receiving benzamil into the kidney showed higher mean BP values than vehicle (88±0.3 vs. 97±4 mmHg, p<0.01). We conclude that CTGF participates in TGF resetting and RBF regulation after UNx, and that could participate in BP regulation after UNx.

C.A. Romero: None. S. Monu: None. R. Knight: None. O.A. Carretero: None.
Bilateral Renal Denervation Attenuates Hypertension in Male Rats Deficient of Functional Endothelin B Receptors but Does Not Affect Salt-Sensitivity

Bryan K Becker, Univ of Alabama at Birmingham, Birmingham, AL; Amanda C Feagans, Univ of Evansville, Evansville, IN; Chunhua Jin, David M Pollock, Univ of Alabama at Birmingham, Birmingham, AL

Independent studies of renal sympathetic nerves and the endothelin (ET) system have demonstrated important contributions of each in the progression of hypertension. Very few studies, however, have investigated the interaction between the ET system and renal nerves in relation to blood pressure control and electrolyte homeostasis. Although endothelin B (ETB) receptors in the renal medulla promote natriuresis, ETB receptors on sympathetic neurons are thought to increase neuronal activity. We hypothesized that renal denervation reduces blood pressure in a salt-sensitive, hypertensive model of ET dysfunction, the ETB-deficient (ETB-def) rat, which lacks functional ETB receptors in all tissues except neurons. After bilateral renal sympathetic denervation (Dnx) or sham operation of ETB-def and transgenic control (TG) rats, baseline blood pressure was recorded via telemetry for 5 days on a normal salt (0.49% NaCl) diet followed by a high salt (4.0%) diet. At baseline, ETB-def Dnx rats had a lower 24-hr systolic blood pressure (SBP) (152.6 ± 3.6 mmHg) relative to baseline; p < 0.005). There was a similar increase in SBP in ETB-def Dnx rats (+10.03 ± 2.3 mmHg relative to baseline; p < 0.005), although the ETB-def Dnx group remained lower than ETB-def sham. High salt had no effect on TG sham or Dnx animals (-2.2 ± 1.3 and -0.6 ± 2.8 mmHg relative to baseline). Preliminary evidence from a subset of the animals in this experiment indicated a dramatically reduced inner medullary ET-1 content in ETB-def sham rats vs. TG sham (97.9 ± 15.4 vs. 327.0 ± 25.4 ng/mg total protein; p < 0.005; n = 3-4/group) in both ETB-def and TG groups, Dnx tended to increase inner medullary ET-1 content (181.8 ± 75.8 and 402.7 ± 19.6 ng/mg total protein respectively). We conclude that in a model of ET dysfunction, the renal nerves are integral mediators of hypertension during normal salt diet, but do not mediate the increase in pressure following high salt diet in this model of salt-sensitive hypertension.


Funding: No

Funding Component: P150

Lateral Hypothalamic Leptin and Melanocortin Signaling in the Regulation of Sympathetic Nerve Activity and Blood Pressure

Donal A Morgan, Latisha N McDaniel, Jingwei Jiang, Kenji Saito, Kevin C Davis, Michael Lutter, Kamal Rahmouni, Huxing Cui, Univ of Iowa Carver Coll of Med, Iowa City, IA

Obesity is associated with increased sympathetic nerve activity (SNA), which contributes to the development of hypertension. Hypothalamus plays a fundamental role in both body weight homeostasis and sympathetic outflow, but the underlying neural basis of this association

Funding: No

Funding Component: P149
remains incompletely understood. Leptin and melanocortin systems in the brain are important regulators of body weight homeostasis, SNA and blood pressure. However, the neural circuits by which leptin and melanocortin regulate SNA and blood pressure remain unclear. We have previously shown that both leptin receptor (LepR) and melanocortin 4 receptor (MC4R) are co-expressed in a unique subset of GABAergic neurons in the lateral hypothalamic area (LHA). Because the LHA is a well-known site for SNA and cardiovascular function, we hypothesized that LepR and MC4R signaling in the LHA may play an important role in SNA and blood pressure. Here we show that direct microinfusion of leptin into the LHA increases renal SNA in dose-dependent manner (% changes from baseline at 4th hour: Vehicle - 25.03 ± 7.09 %, 0.05 ug leptin 26.01 ± 9.5 %, 0.5 ug leptin 100.23 ± 26.94 %, n=5-7/group, p<0.001). Additionally, in vivo Cre/loxP system-mediated re-expression of endogenous MC4Rs in the LHA restores the blunted response of MTII-induced increase in renal SNA (Wild-type 66.67 ± 16.53 %, MC4R-null 17.65 ± 10.3 %, MC4R reactivation 48.77 ± 9.36 %, n=7-9/group, p<0.01) and elevates blood pressure in obese, but normotensive MC4R-TB mice in inactive light cycle (Wild-type 111.62 ± 4.07, MC4R-TB 115.75 ± 1.27, MC4R re-expression 125.29 ± 3014 mmHg, n=3-4/group, p<0.05) without significantly affecting body weight. Finally, optogenetic stimulation of LHA LepR-positive neurons decease blood pressure and renal SNA in conscious mice. In conclusion, our findings identify a novel brain circuit by which leptin and melanocortin signaling regulate renal SNA and blood pressure.


Funding: Yes

Adam17 in the Paraventricular Nucleus Contributes to Activation of Pre-Autonomic Neurons and Maintenance of Baseline Blood Pressure

Snigdha Mukerjee, LSU Health, New Orleans, LA; Andrea Zsombok, Tulane Univ, New Orleans, LA; Eric Lazartigues, LSU Health, New Orleans, LA

We previously reported that over-activation of the classical arm (i.e. ACE/Ang-II/AT1 receptor) of the brain renin-angiotensin system (RAS) up-regulates ADAM17, a disintegrin and metalloprotease. ADAM17 cleaves angiotensin converting enzyme 2 (ACE2) from the cell surface, leading to a compromised compensatory RAS axis (i.e. ACE2/Ang-(1-7)/Mas receptor) and neurogenic hypertension. We hypothesized that ADAM17 within the paraventricular nucleus of the hypothalamus (PVN) intrinsically regulate neuronal activity, thus contributing to sustained sympathetic drive and the development of neurogenic hypertension. To test this hypothesis, a new transgenic mouse line, named SAT, was generated to selectively knockdown ADAM17 in pre-autonomic Sim1 neurons within the PVN, using cre-loxP technology. Mean arterial pressure recorded in conscious mice (telemetry) showed a significant reduction at baseline (ΔMAP: -8 ±2 mmHg, N=3, P<0.05) and an increased vagal tone (Heart rate: 231 ±19 vs. 160 ±11 bpm, N=10, P<0.05) in SAT vs. control (NT) litter-mates. To address the impact of ADAM17 deletion on neuronal excitability, c-Fos immunohistochemistry was performed. SAT mice exhibited a significant reduction of c-Fos

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

P151
expression in the PVN compared to NT (14 ±2 vs. 124 ±8 neurons, N=3, P<0.001). Retrograde labeling (pseudorabies virus-eGFP) confirmed that a sub-population of these neurons projected to the kidney. In addition, mRNA expression of the GABAa receptor was higher in the hypothalamus of SAT compared to NT mice (1.3 ±0.1 vs. 1.0 ±0.1, N=7, P<0.05). ADAM17 knockdown from the PVN had a positive effect on the compensatory RAS, as evidenced by a rise in hypothalamic ACE2 activity compared to NT mice (82.3 ±4.2 vs. 69.7 ±3.3, N=7, P<0.05). Interestingly, this was also extended downstream of the PVN, with an increase in ACE2 activity (96.7 ±3.8 vs. 82.0 ±3.4, N=7, P<0.05), as well as Mas receptor gene expression (0.28 ±0.03 vs. 0.17 ± 0.01, N=6, P<0.01) in the brainstem of SAT compared to NT mice. Altogether, our data highlight a new role for ADAM17 in neuronal excitability and the maintenance of baseline blood pressure. Potential mechanisms opposing ADAM17-mediated neuronal excitability of PVN neurons include GABAergic pathways and the compensatory axis of the brain RAS.

S. Mukerjee: None. A. Zsombok: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; HL093178. E. Lazartigues: A. Employment; Modest; HL093178. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; HL093178.

Funding: Yes
Funding Component: National Center
P152

**Aminopeptidase A in the Brain Exerts Cardiovascular Action via Degradation of Kallidin to Bradykinin**

Takuto Nakamura, Masanobu Yamazato, Yusuke Ohya, Univ of the Ryukyus Sch of Med, Okinawa-Ken Nakagami-Gun Nishihara-Cho, Japan

Objective: Aminopeptidase A (APA) degrades various sympathomodulatory peptides such as angiotensin (Ang) II, cholecystokinin-8, neurokinin B and kallidin. APA activity is increased in the brain of hypertensive rats. A centrally acting APA inhibitor produg is currently under investigation in clinical trial for treatment of hypertension. In previous reports, a role of APA in the brain on cardiovascular regulation was researched focus on only renin-angiotensin system. We previously reported that intracerebroventricular(icv) administration of APA increased blood pressure and that this pressor response was partially blocked by angiotensin receptor blocker. In this study, we evaluated a role of APA on cardiovascular regulation focusing on peptides other than Ang II. Method: Eleven weeks old Wistar Kyoto rats were used. We icv administrated 800 ng/8 μL of APA after pretreatment of following drugs, i) 8μL of artificial cerebrospinal fluid (aCSF) as a control, ii) 80 nmol/8 μL of amastatin which is a non-specific aminopeptidase inhibitor, iii) 1 nmol/8 μL of HOE-140 which is a bradykinin receptor blocker to evaluate the involvement of degradation of kallidin to bradykinin by APA. Result: i) Icv administration of APA after pretreatment of aCSF increased blood pressure rapidly. Blood pressure reached a peak within 1 minute. The elevated blood pressure decreased gradually and reached baseline blood pressure in 10 minutes. A peak pressor response is 25.5±1.4 mmHg (n=5). ii) Icv pretreatment of amastatin or HOE-140 did not change the blood pressure. A peak pressor response induced by APA is 13.1±4.1 mmHg (n=6, p<0.05 vs aCSF). iii) Icv pretreatment of HOE-140 did not change the blood pressure. A peak pressor response induced by APA is 21.2±1.8 mmHg (n=4, p<0.05
vs aCSF). Conclusion: 1) Icv administration of APA increased blood pressure by APA enzymatic activity. 2) Cardiovascular regulation of APA in the brain is due to not only degradation of Ang II to Ang III but also degradation of kallidin to bradykinin. Clinical implication: We think inhibition of APA in the brain may be a unique therapeutic target which affects several cardiovascular peptides in the brain.

**T. Nakamura:** None. **M. Yamazato:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; MSD. **Y. Ohya:** None.

**Funding:** No

**Funding Component:** P153

**Downregulation of Neuronal Nitric Oxide Synthase Within the Paraventricular Nucleus in Insulin Dependent Diabetic Akita Mice**

**Neeru M Sharma,** Paras K Mishra, Kaushik P Patel, UNMC, Omaha, NE

Activation of both renin-angiotensin- system (RAS) and sympathetic system are the primary etiologic events in the development of hypertension in diabetes mellitus (DM). However, the precise mechanisms for sympathetic activation in DM have not been elucidated. Our previous studies have demonstrated that neuronal nitric oxide (nNOS) expression and nitric oxide (NO) mediated inhibition of sympathetic nerve activity (SNA) is markedly reduced in the paraventricular nucleus (PVN) of streptozotocin-induced diabetic rats. We have further demonstrated that Angiotensin II (Ang II) via Ang II type 1 receptors (AT1R) modulates the expression of nNOS in the PVN, which augments sympathetic outflow. Here we hypothesized that DM-linked hypertension and cardiovascular dysregulation is due to the reduction in nNOS with the PVN.

To test the hypothesis, we used Ins2+/− Akita (a spontaneous, insulin dependent genetic diabetic murine model) which showed an increase in systolic blood pressure at the age of 14 weeks compared to corresponding C57BL/6J (WT) mice with concomitant decreased expression of nNOS (0.75±0.05 WT vs. 0.43±0.11* Akita) in the PVN. Further, Akita mice had increased expression of ACE (angiotensin converting enzyme) (WT 0.34±0.04 vs. Akita 0.58±0.05*) and AT1R (WT 0.29±0.09 vs. Akita 0.49±0.03*) and decreased expression of ACE2 (0.27±0.03 WT vs. 0.17±0.05* Akita) and Mas receptor (WT 0.77±0.07 vs. Akita 0.46±0.02*), suggesting an imbalance in the excitatory and protective arms of RAS. Moreover, we found increased protein levels of PIN (a protein inhibitor of nNOS, known to dissociate catalytically active nNOS dimers to monomers) (WT 0.71±0.09 vs. Akita 1.75±0.08) with 72 percent decrease in dimer/monomer ratio of nNOS (WT 0.19±0.0 vs. Akita 0.11±0.04) in the PVN of Akita mice. Taken together, our studies suggest that accumulation of PIN, mediated by activation of the excitatory arm of RAS, leads to a decrease in the active dimeric form of nNOS resulting in reduced NO causing an over-activation of the sympathetic drive, leading to hypertension in DM.

**N.M. Sharma:** None. **P.K. Mishra:** None. **K.P. Patel:** None.

**Funding:** Yes

**Funding Component:** National Center P154

**Hypertension in Obese African American Women is Not Caused by Increased Sympathetic Activity**

**Cyndya A Shibao,** Vanderbilt Univ Medical Ctr, Nashville, TN; Alejandro Marinos, Beaumont Medical Ctr, Michigan, IL; Jorge E Celedonio,
African American (AA) women have the highest prevalence of hypertension and obesity in the United States. We tested the hypothesis that sympathetic activity contributes to hypertension in obese AA women, as we previously shown to be the case in Caucasians. We studied 42 obese women (16 whites, body mass index (BMI) 36± 4 kg/m², 44% with diagnosis of hypertension (HTN) and 26 AA, BMI 35± 4 kg/m², 46% HTN). Anti-HTN medications were discontinued for 2 weeks prior to the study day. All subjects underwent complete autonomic blockade with the ganglionic blockade trimethaphan at doses of 4 mg/min. Autonomic blockade was evaluated by the lack of heart rate changes in response to ~25 mm Hg increase in blood pressure produced by a bolus infusion of the alpha 1 adrenergic agonist, phenylephrine and the decrease in norepinephrine levels. Results: Plasma norepinephrine significantly decreased during trimethaphan infusion (from baseline 253±1107 to 61±29 pg/ml, trimethaphan). The decrease in mean arterial blood pressure (MAP) produced by trimethaphan was greater in obese HTN compared with normotensive (NTN) Caucasians (-27±10 vs. -15±8 mm Hg, P=0.016). In contrast, no difference in the decrease in MAP induced by trimethaphan was found between HTN and NTN obese AA women (-16±11 vs. -12±10, P=0.451, figure). Heart rate increased similarly with trimethaphan between HTN and NTN Caucasians (+9.1± 6 vs. 16± 9, P=0.109) and AA women (+22± 7 vs. 21±12 bpm, P=0.760). MAP remained elevated in HTN obese AA women during trimethaphan infusion (84±15 vs. 72±9.8 mm Hg in NTN AA, P=0.021). Conclusion: Sympathetic activity does not contribute to hypertension in AA women.
Introduction: Gut dysbiosis has been linked to hypertension in both rodents and humans. Microbial metabolites such as propionate have been shown to regulate blood pressure (BP), while butyrate, one of the major fermented end-products of fiber, reportedly produces beneficial anti-inflammatory effects in multiple dysbiosis-related diseases. Therefore, we tested the impact of a fiber-rich, butyrolytic diet on BP regulation and immune responses in the spontaneously hypertensive rats (SHR).

Methods: SHR (5 wo) were placed on either the fructooligosaccharides/inulin-rich diet (Fiber, N=6), or its calorie-matched control diet (Control, N=6) (Research Diets, Inc.) for 10 weeks. Baseline BP was measured by tail cuff every week for the duration of the study. Fecal samples were collected for HPLC analysis of butyrate, and \textit{Lactobacillus} population by QPCR. Manganese-enhanced magnetic resonance imaging was used to monitor neural activity in cardioregulatory brain regions. Blood was analyzed for circulating lymphocyte populations previously implicated in BP control in the SHR (CD3+CD45+, CD4+CD25+, CD8+).

Results: Fiber-rich diet produced an increase in fecal butyrate levels as early as five weeks (Control vs. Fiber, 4.9umol/g vs. 9.7umol/g, p=0.068, N=6). This was associated with contraction of fecal \textit{Lactobacillus} (47.5% vs. 5.9%, p=0.0008, N=6). However, we observed significantly higher systolic BP (181.4mmHg vs. 201.7 mmHg, p=0.0088, N=6) in the fiber group compared with control, beginning with week 9 post-diet switch. Changes in neural activation were observed in the paraventricular nucleus of hypothalamus (PVN) (3.3 voxel vs. 7.3 voxel, p=0.26) and amygdala (93.3 voxel vs. 31 voxel, p=0.0059, N=3). No changes in circulating T-lymphocytes were observed between the two groups: CD3+CD45+ (32.4% vs. 32.1% lymphocytes); CD4+CD25+ (1.05% vs. 0.78% lymphocytes); CD8+ (18.7% vs. 16.2% lymphocytes, N=6) at week 10 post-diet switch.

Conclusion: Fiber-rich diet suppression of \textit{Lactobacillus} is associated with increase in BP in the SHR, independently of T-lymphocyte responses. The observed higher neural activity in PVN and lower in amygdala in the fiber group suggest direct effects of gut bacterial metabolites on brain cardioregulatory regions.


Funding: Yes
Funding Component: National Center
P157

Assessment of Vascular Endothelial Function in Postural Tachycardia Syndrome and Healthy Controls


Postural tachycardia syndrome (POTS) is a heterogeneous disorder characterized by an excessive rise in HR and symptoms consistent with cerebral hypoperfusion while upright. Increased sympathetic activity may contribute to this condition and may also impair nitric oxide (NO)-function. We evaluated NO function using flow-mediated dilation (FMD) and Peripheral Artery Tonometry (PAT) in POTS patients and age-matched controls. We studied 16 POTS patients (30±2 years, BMI 22.3±1 kg/m²) and 7 healthy control subjects (HC; 31±2
years, BMI 22.1±1 kg/m²). Medications affecting BP, blood volume, the immune system, and autonomic function, were withheld for ≥5 half-lives. All subjects followed the same low-monoamine, caffeine-free diet for ≥3 days before testing. Endothelial function was measured as the percentage change in FMD (%FMD) and using the reactive hyperemic index (RHI) for PAT. We also measured autonomic function, plasma levels of catecholamines, renin activity (PRA) and aldosterone. We found that POTS patients had a significantly blunted FMD (6.11±0.8 vs. 9.67±1.6 %, P=0.049, figure), compared to healthy controls. This blunted FMD response was similar as what our group has reported in obese hypertensive females (N=13, 5.7±0.9%, figure). Also, as expected they had higher upright HR (121±6 vs. 90±6 bpm, for POTS and HC, P=0.020). There were no differences in PAT (2.08±0.12, vs. 1.8±0.13 RHI, for POTS and HC, P=0.168). There were no differences in norepinephrine (765±150 vs. 545±39 pg/mL, P=0.955), renin activity (5.3±1.3 vs. 5.2±1.6 ng/mL/hr, P=0.735) or aldosterone (19.6±3.8 vs. 15.9±5.5 ng/dL, P=0.129).


Funding: No
Funding Component: P158

Cullin3 Regulated Endothelial Function by Modulating eNOS Activity

Jing Wu, Ko-Ting Lu, Larry N. Agbor, Chunyan Hu, Xuebo Liu, Masashi Mukohda, Anand R. Nair, Curt D Sigmund, Univ of Iowa Carver Coll of Med, Iowa City, IA

Pseudohypoaldosteronism type II (PHAII) patients expressing dominant negative cullin3 mutations exhibit increased renal NaCl reabsorption and develop hyperkalaemia, metabolic acidosis and hypertension. It is unclear whether loss of cullin3 function in extra-renal tissues contributes to the hypertensive phenotype. In the vasculature, endothelial Nrf2 stability is tightly regulated by cullin3-based E3 ubiquitin ligase via the redox-sensitive adaptor kelch-like ECH-associated protein 1. In the present study, we found that 24-hour treatment with a pan cullin inhibitor MLN4924 (1 μM) caused a 3-fold increase in Nrf2 protein in mouse lung endothelial cells (MLECs), while tert-butyl hydroperoxide (tBHP, 240 μM) had no effect on Nrf2 level. However, both MLN4924 and tBHP triggered time-dependent accumulation of Nrf2 in the nuclei, which peaked at 40 minutes following treatment. As a result, both treatments induced marked upregulation of antioxidant genes including NAD(P)H quinone oxidoreductase 1, heme oxygenase 1, glutamate cysteine ligase (rate-limiting enzyme in glutathione synthesis), and catalase both in MLECs and primary mouse aortic endothelial cells (MAECs). Of note, MLN4924 upregulated Nox4 expression (1.0 ± 0.15 vs 1.7 ± 0.2) and tBHP upregulated Nox1 (1.0 ± 0.2 vs 4.8 ± 1.1), while MLN4924 and tBHP both markedly increased intracellular
superoxide as determined by dihydroethidium staining. In addition, intracellular nitric oxide was decreased by half in MLN4924-treated MLECs. This redox imbalance was likely due to impaired eNOS expression and activation as MLN4924 caused a 25% reduction in total eNOS and a 75% reduction in phosphorylated eNOS, while tBHP lead to a 50% reduction in phosphorylated eNOS with no effect on total eNOS. This suggests that decreased eNOS activation contributed to the oxidative stress induced by these agents. These data imply that suppression of cullin3 in arterial endothelial cells may dampen endothelium-dependent vascular relaxation and contribute to the blood pressure elevation observed in PHAII patients with global loss of cullin3 function. Although cullin3 also negatively regulates Nrf2-mediated antioxidant responses in vascular endothelial cells, this likely occurs as a compensatory mechanism.

J. Wu: None. K. Lu: None. L.N. Agbor: None. C. Hu: None. X. Liu: None. M. Mukohda: None. A.R. Nair: None. C.D. Sigmund: A. Employment; Significant; University of Iowa. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA SFRN. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Significant; Carver Trust.

Funding: Yes
Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

P159

Scavengers of Isolevuglandins as Novel Therapeutic Approaches for Treating Hypertension & Associated Inflammation

Wei Chen, Liang Xiao, Annet Kirabo, Danielle L Michell, Sean S Davies, Venkataraman Amarnath, Jackson L Roberts, David Harrison, Vanderbilt, Nashville, TN

Adaptive immunity and especially T lymphocytes play a crucial role in the development of hypertension. Proteins that are oxidatively modified by highly reactive isolevuglandins (isoLGs) accumulate in dendritic cells & lead to subsequent activation of T lymphocytes. This process can be prevented by compounds known to scavenge isoLGs, such as 2-hydroxybenzylamine (2-HOBA). The overall goal of current study is to develop novel antihypertensive drugs based on this isoLG scavenging strategy, that will not only prevent but will also reverse hypertension and its associated inflammatory end-organ damage. Initially, the preventative effects of 10 putative isoLG scavengers were tested. C57Bl/6 mice received angiotensin (Ang) II (490 ng/kg/min) infusion for 14 days. Each compound was administered in the drinking water (1mg/ml) at the onset of Ang II infusion for 7 days to a half of the mice & for entire 14 day period to another half of the animals. Blood pressure was measured by tail cuff method. Among 10 compounds, we found that 2-HOBA, methyl 2HOBA (Me2HOBA) & 3-methoxy2-HOBA (3-Mo2HOBA) were most effective in lowering blood pressure. Interestingly, the pyridoxamine analogs were not effectively lowered blood pressure. We further examined the efficacy of our 3 most effective compounds in reversing established hypertension. Mice received Ang II infusion for 6 weeks and received each study drug (2 mg/ml) during the last 4 weeks. We employed radiotelemetry to monitor blood pressure. Compared with untreated mice, Me2-HOBA, 3-Mo2-HOBA & 2-HOBA were equally effective in lowering blood pressure by 20 mmHg (all p < 0.05 vs no drug). Six weeks of Ang II infusion caused 2- to 4-fold increases in renal
T cell (CD3+) & monocyte/macrophage (F4/80+) infiltration as measured by immunohistochemistry & all three compounds markedly reduced these by 50%. These treatments also reduced aortic fibrosis as measured by Masson’s Trichrome blue staining. We conclude that these isoLG scavengers can be potentially used as new effective therapy for lowering blood pressure & attenuating hypertensive renal & vascular damage. Variability of in vivo effectiveness for the different scavengers likely reflects differences in bioavailability due to structure & lyophilicity.


Funding: No
Funding Component: P160

Non-invasive Method to Assess Human Circadian Rhythms

Daian Chen, S Justin Thomas, David A Calhoun, David M Pollock, Jennifer S Pollock, The Univ of Alabama at Birmingham, Birmingham, AL

Circadian rhythms are controlled by an endogenous time-keeping system oscillating approximately on a 24-h cycle under constant conditions. These rhythms depend on a network of interacting genes and proteins, including transcriptional activators such as CLOCK, NPAS2, and ARNTL (BMAL1), which induce transcription of the clock genes Period (Per1, Per2, and Per3) and Cryptochrome (Cry1 and Cry2). Human salivary cortisol and melatonin follow a clear circadian rhythm as well. Disruption of the circadian rhythm and sleep-wake cycles are considered risk factors for a variety of health problems, especially hypertension and other cardiovascular and metabolic diseases. Here we put together practical methods for assessing circadian rhythms in adult subjects conducted by each individual. This method is non-invasive, inexpensive and provides a predictive profile of an individual’s circadian rhythm related to clock-controlled gene expression in buccal cells, salivary cortisol, salivary melatonin, and subject’s activity or sleep. Subjects are instructed on how to obtain buccal cells using swabs (Whatman OmniSwab) from the inside of their cheeks and collect saliva using salivettes (Sarstedt) every 4 hours starting at 6am, for 2 consecutive days. Subjects also wear actigraphy watches (Phillips Respironics) during the 2 days, to record their activity, light exposure and estimates of sleep times. To monitor adherence to correct time point collections, each subject is given an electronic vial called eCAP (Information Mediary Corp) that records the exact time the container is opened to place samples once collected. We demonstrate feasibility to extract up to 150ng/µl of RNA (Ambion RNAqueous-Micro Total RNA Isolation Kit) from buccal cells swabs. Salivary melatonin and cortisol are measured by radioimmunoassay (Buhlmann Lab) with melatonin peak levels ranging from 14 to 23 pg/ml and cortisol peak levels ranging from 10 to 24 ng/ml. We suggest that buccal cell expression of clock-controlled genes, salivary melatonin, salivary cortisol, and actigraphy data are valuable in providing reliable assessment of human circadian rhythm profiles under a variety of conditions.


Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)
P161

Inspiratory Muscle Training and Aerobic Training in the Treatment of Hypertension:
Baroreflex Sensitivity, Sympathetic Activity and Endothelial Function Responses

Janaina B Ferreira, Valéria Hong, Otávio Coelho, Silvia Cavasin, Fernando Santos, Fernanda Consolim-Colombo, Maria Cláudia C Irigoyen, Heart Inst Med Sch Sao Paulo Univ, Sao Paulo, Brazil

Arterial hypertension is associated to sympathetic hyperactivity and endothelial dysfunction. Aerobic training (AT) is highly recommended to improve vascular function minimizing complications and Inspiratory muscle training (IMT) has demonstrated beneficial effects in this population, especially improving cardiovascular autonomic control. We sought to observe the effects of both training modalities on baroreflex sensitivity, sympathetic activity and endothelial function, in patients with controlled arterial hypertension. 10 patients (55±4 years old, both genders) were included and allocated into two groups: IMT (n=5, 7days/week, 30min/day, load=30%PImax) and AT (n=5, 2days/week, 1hour/day, load=70%HRmax). Both training protocols were performed during 12 weeks. Blood pressure (BP) and heart rate (HR) signals were recorded before and after protocols, as well as the other evaluations, by pulse telemetry (Finometer®PRO) and ECG (PowerLab®). Arterial baroreflex sensitivity was analysed by sequence method. Sympathetic activity was evaluated by microneurography (PowerLab®) and the endothelial function was evaluated by flow mediated dilation (EnVisor CHD, Philips, Bothell, WA, USA). After 12 weeks treatment IMT improved baroreflex sensitivity to both tachycardic and bradycardic responses respectively (BRR Down Gain (mean): IMT=26.51(±1.7)vs15.57(±6.7), AT=13.94(±5.5)vs17.92(±1.6); BRR Up Gain (mean): IMT=17.16(±1.2)vs16.28(±1.1), AT=12.39(±5)vs12.69(±3.3)). Additionally, we observed reduction of sympathetic activity in both groups (IMT:33.23±11.79vs25.07±13.28; AT:29.88±7.07vs24.09±6.37) and improvement of endothelial function independent of the treatment (IMT:6.4±2.18vs7.22±2.08; AT:5.49±7.43vs7.06±3.12). Regarding the responses to inspiratory muscle training and aerobic training on autonomic cardiovascular control and endothelial function in Hypertension, we demonstrated for the first time that IMT and AT present quite similar effects in patients with controlled blood pressure.

J.B. Ferreira: None. V. Hong: None. O. Coelho: None. S. Cavasin: None. F. Santos: None. F. Consolim-Colombo: None. M.C. Irigoyen: None.

Funding: No
Funding Component: P162

Butyrate, a Microbial Metabolite, Attenuates Angiotensin II-induced Hypertension and Gut Dysbiosis

Seungbum Kim, Gilberto O. Lobaton, Mohan K. Raizada, Univ of Florida, Gainesville, FL

Objectives: We have previously established that hypertension (HTN) is associated with gut dysbiosis in rat models and patients with high blood pressure. Gut dysbiosis was associated with an increase in Firmicutes/Bacteriodetes ratio and a decrease in butyrate-producing microbial populations. This led us to hypothesize that infusion of butyrate would overcome gut microbial dysbiosis induced butyrate deficiency and reverse angiotensin II (Ang II)-induced HTN. Design and Method: Four groups of C57BL6 mice were infused with either saline, Ang II (1000ng/kg/min), Ang II + sodium butyrate (1mg/kg), or sodium butyrate alone for 4 weeks. Fecal samples were analyzed by 16S bacterial rDNA sequencing for gut microbiome identification. Intestinal leukocytes were
analyzed using FACS. **Results:** Ang II induced increase in MAP was significantly attenuated in mice co-administrated with butyrate (Control, 102.1±5.7 mmHg; Ang II, 148.3±8.1 mmHg; Ang II+Butyrate, 120.5±11.2 mmHg). Microbiota analysis demonstrated a significant increase of gut dysbiosis in Ang II-HTN that was normalized by butyrate treatment (F/B ratio: Control, 2.6; Ang II, 5.4; Ang II+Butyrate, 2.0). Principal Coordinates Analysis indicated each group in the input phylogenetic tree had significantly different microbial communities. LEfSe analysis showed that there were decreases of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, and increases of *Corynebacterium* and *Staphylococcus* at the genus level of Ang II-HTN mice. Furthermore, inflammatory status of the gut as evidenced by the level of mucosal T cells in lamina propria from these groups showed that there was an increase of CCR2+ Th17 cells in Ang II-HTN mice, but not in butyrate co-treated mice (Control, 15.7%; Ang II, 28.4%; Ang II+Butyrate, 15%).

**Conclusions:** These observations show that gut dysbiosis and the decrease of butyrate producing bacteria are associated with Ang II-HTN. Thus, supplementing butyrate in Ang II treated mice attenuated HTN and reversed gut dysbiosis, as well as normalizing the intestinal Th17. These data suggest butyrate producing bacteria could be considered as a novel probiotic therapy for hypertension.


Funding: No

Funding Component: P163

**Effects of Erythropoietin and Erythropoietin-receptor Materials in Blood Pressure, Hematocrit and Body- Spleen Weight Variability**

Mary S Lee, Northwestern Univ Feinberg Sch of Med & Univ of MN Sch of Med, Chicago/Minneapolis, IL; John S Lee, Princeton Univ & Univ of MN Sch of Med, Princeton/Minneapolis, NJ; Jong Y Lee, Franz Halberg, Univ of MN Sch of Med, Minneapolis, MN

Adversity with erythropoietin (Epo) treatment (Epo-Rx) is grim in the cardio-cerebrovascular system. Because circadian rhythmic variability is an important aspect of cardiovascular function, we evaluated circadian blood pressure (BP), hematocrit (Hct), body and spleen weight (BW, SW) variability immediately before and after 4-week, twice-weekly course of Epo-Rx (50 U/kg) along with physiological saline (control), pure human Epo-receptor protein (Epo-bp) and anti-Epo-bp antibody (αEpo-bp) groups at midnight, 4 AM, 8 AM, noon, 4 PM and 8 PM in Sprague-Dawley rats.

In our earlier report, Epo-Rx increased BP, Hct and SW markedly overall compared with saline, Epo-bp, and αEpo-bp groups (Hypertension 2007;50:439-45). In this current report, variability (%) of BP, Hct, BW and SW are compared. Epo-bp treated BP Variability (BPV) was lower than others (9.5 vs. 15.5 control and others in similar values). Phase changes were noticed when exposed to Epo (daytime peaks in Epo, Epo+Epo-bp and Epo+αEpo-bp). Hct variability (HctV) in Epo and Epo-bp treated groups shows somewhat lower than in control group (18.3 in C vs. 13.3, 12.9, and 19.4 in Epo, Epo-bp and αEpo-bp, respectively). However, Epo effect was additive to HctV when Epo was added to Epo-bp or αEpo-bp (32.0 and 30.4, respectively). All 5-study groups showed phase changes, although Epo-exposed groups had a much greater magnitude with markedly increased Hct (peak at 19:00 in control and 5 study groups during the daytime through noon-hour peaks). BW variability is similar in each group (except lower BW in the Epo-group) with
various peak hours but SW variability (SWV) showed a similar pattern as shown in HctV. Best treatment times for Epo, Epo+Epo-bp and Epo+αEpo-bp were estimated: BP at 4 PM, 4 AM and 4 AM, respectively; Hct at 4 AM and both at midnight, respectively; SW at midnight, 8 PM and noon, respectively. Thus, significantly increased BPV, HctV and SWV in Epo-Rx than those of other groups and extremely increased Hct resulted in splenomegaly.

M.S. Lee: None. J.S. Lee: None. J.Y. Lee: None. F. Halberg: None.

Funding: No
Funding Component: P164

The Role of the Adrenoreceptors Beta 3 on Metabolic Syndrome Induced by Fructose

Eduardo Dias Jr., Alexandre Tavolari, Marina Souza, Renata Oliveira, Patricia Fiorino, Miriam O Ribeiro, Vera M Farah, Univ Presbiteriana Mackenzie, São Paulo, Brazil

The aim of this study was to evaluate the role of the adrenoreceptor beta 3 (ARβ3) in the cardiovascular function on Metabolic Syndrome (MS) induced by fructose overload. Male mice after weaning (8-10g) were divided into 4 groups (n=7/group): FVB Control (C) and ARβ3 Knockout Control (Kβ3C), with free access to food and water for 8 weeks; FVB fructose (F) and ARβ3 Knockout fructose (Kβ3F), with free access to food and fructose added to the drinking water (10%) for 8 weeks. At the end of the protocol, intraperitoneal glucose tolerance test was performed. Triglycerides and HDL fractions were evaluated by colorimetric methods. The animals were submitted to catheterization of carotid artery. This catheter was connected to a transducer and continuous signals of blood pressure (BP) was recorded. The variability of the resultant signal was evaluated in frequency domain. Visceral fat deposits were collected, weighted and normalized as fat (mg)/body weight (g). Results: fructose increased the fasting glycemia in the knockout group (Kβ3C=93±3 vs. Kβ3F=122±7 mg/dL) and decreased the glucose tolerance in both fructose groups. Fructose increased the triglycerides and decreased HDL cholesterol. The uric acid increased in the knockout animals. There was an increase on visceral fat deposits only in FVB animals (C=10±2 vs. F=16±1 mg/g), as well as on blood pressure (C=123±4 vs. 136±2 mmHg). There was an increase on blood pressure variability for all experimental groups compared to Control (C=10.7±1 vs. F=36±11; Kβ3C=50±7; Kβ3F=24±4 mmHg²) as well as on peripheral sympathetic modulation (C=3.3±0.4 vs. F=8.1±0.1; Kβ3C=22.9±3; Kβ3F=9.2±2 mmHg²). Furthermore, Kβ3F showed a decrease on blood pressure variability and peripheral sympathetic modulation. Our data suggest that the hypertension induced by fructose is related with adiposity. Moreover, the blood pressure variability and its sympathetic modulation are dependent of the ARB3. The association between fructose and ARB3 knockout leads to the loss of the sympathetic modulation.


Funding: No
Funding Component: P165

Exploration of the Mechanism in Which Saccharina Japonica Alleviates an Increase in Blood Pressure in Renovascular Hypertensive Rats

Saki Maruyama, Yukiko Segawa, Kobe Women's Univ, Suma, Kobe, Japan; Hiroko Hashimoto, Osaka Seikei Junior Coll, Higashiyodogawa, Osaka, Japan; Tomoko Osea, Nobutaka Kurihara, Kobe Women's Univ, Suma, Kobe, Japan
**Objective:** Saccharina japonica (SJ), one of brown algae, is a common foodstuff in Japan and neighbor countries. Some studies have shown that the intake of SJ decreases blood pressure (BP) in spontaneously hypertensive rats. As well, we previously observed it in 2-kidney, 1-clip renovascular hypertensive (2K1C) rats. However, the mechanism is still unclear. One of possible components of SJ which play an important role in decreasing BP is alginate. Since alginate is richer in the roots than in the blades in SJ, in the present study, we compared the effects in alleviating BP of intake of SJ roots with that of SJ blades in 2K1C rats. We also evaluated angiotensin II receptor type 1 (AT1R) mRNA to investigate the role of renin-angiotensin system in the mechanism.

**Methods:** Male Sprague-Dawley rats (6 wks) were treated with sham operation (SHAM) or clipping the left renal artery (2K1C). After surgery, the rats started receiving a control diet (C) or a diet containing 5.0% (w/w) SJ blades (B), or SJ roots (R) for 6 weeks. The systolic BP (SBP) was measured by a tail-cuff method every week. At the end of the protocol, mean arterial pressure (MAP) was measured in each rat under anesthesia. Then, the aortas were removed for extracting mRNA. AT1R mRNA expression was evaluated using reverse transcriptase quantitative real-time PCR.

**Results:** SBP was significantly higher in 2K1C-C than SHAM-C through the experiment period (p<0.001). SBP in 2K1C-B and -R was significantly lower than in 2K1C-C (p<0.001). 2K1C-B showed a significant reduction in SBP compared with in 2K1C-R (p<0.05). At the end of the protocol, MAP showed the similar trend to SBP. AT1R mRNA expression was higher in 2K1C than in SHAM, but there were no significant differences among 2K1C-C, -B and -R.

**Conclusion:** Although alginate is richer in the roots than in the blades in SJ, the effects in alleviating BP was higher in the blades than in the roots. Thus, alginate may play no major role in the mechanism. AT1R may not play an important role, neither. Therefore, we need investigate other possible mechanisms.

**S. Maruyama:** None.  **Y. Segawa:** None.  **H. Hashimoto:** None.  **T. Osea:** None.  **N. Kurihara:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Grant- in-Aid for Scientific Research, #15K00862.

**Funding:** No

**Funding Component:** P166

The Effect of a Muscadine Grape Extract on Angiotensin II-Induced Hypertension and Cardiac Damage


Muscadine grapes, indigenous to the southeastern United States, are a dietary source of polyphenols, such as ellagic acid, quercetin, and resveratrol, which have both anti-inflammatory and antioxidant properties. Preclinical studies show that ground muscadine grape skins and seeds have anti-cancer effects, suggesting that supplementation with a muscadine grape extract (MGE) may serve as a chemotherapeutic or chemopreventive agent. As cancer patients often present with hypertension, diabetes, and other vascular pathologies, it is imperative to determine the effect of a muscadine grape supplement on these underlying comorbidities. The goal of this study was to determine whether MGE administered in the drinking water will exacerbate or improve hypertension and cardiac damage. Sprague-Dawley rats receiving a 4-week infusion of Ang II via subcutaneous osmotic mini-pump (24 µg/kg/h) had a significant increase in systolic blood pressure.
(123.1 ± 2.3 mm Hg in control vs. 213.1 ± 6.8 mm Hg in Ang II-treated; n=8; p < 0.0001). MGE supplementation had no effect on blood pressure or gross cardiac hypertrophy in either normotensive or hypertensive rats. Additionally, MGE did not alter stroke volume or cardiac output, measured by VEVO ultrasound. However, MGE ameliorated the Ang-II induced decrease in diastolic function, as measured by the E/E’ ratio (19.9 ± 0.8 in control, 28.1 ± 1.1 in Ang II-treated rats, 22.3 ± 2.0 in Ang II/MGE-treated rats; p < 0.05). Co-treatment with MGE also significantly reduced the Ang II-mediated increase in cardiomyocyte cross-sectional area (340.5 ± 12.0 µm² in control, 423.2 ± 17.1 µm² in Ang II-treated rats, and 342.6 ± 13.2 µm² in Ang II/MGE-treated rats, p < 0.01). MGE supplementation of Ang II-treated rats decreased interstitial cardiac fibrosis, measured by Picrosirius red staining (1.0 ± 0.1% in control, 2.0 ± 0.2% in Ang II-treated rats and 1.4 ± 0.1% in Ang II/MGE-treated rats, p < 0.05). These results demonstrate that treatment with MGE does not exacerbate hypertension or hypertension-induced cardiac damage but ameliorates cardiac hypertrophy, suggesting that MGE supplementation has an acceptable safety profile for use as an anti-cancer agent in hypertensive patients and may be used to treat cardiac hypertrophy and damage.


Funding: No
Funding Component: P167

Combined Exercise Training is Better than Isolated Aerobic and Resistance Exercise Training for an Experimental Model of Metabolic Syndrome and Menopausal Rats

Filipe F Conti, Janaina O Brito, Univ Nove de Julho, São Paulo, Brazil; Nathalia Bernardes, Univ de São Paulo/Heart Inst, São Paulo, Brazil; Danielle S Dias, Univ Nove de Julho, São Paulo, Brazil; Maria C Irigoyen, Univ de São Paulo/Heart Inst, São Paulo, Brazil; Kátia De Angelis, Univ Nove de Julho, São Paulo, Brazil

The aim of this study was to verify the effects of three different moderate exercise training protocols (aerobic, resistance and combined (aerobic + resistance)) in a model of metabolic syndrome and menopause on a cardiovascular parameter and oxidative stress. Female SHR rats were divided into (n=8): hypertensive (H), hypertensive ovariectomized submitted to fructose overload (100g/L in drinking water) (HFO), aerobic trained hypertensive ovariectomized submitted to fructose overload (AHOF), resistance trained hypertensive ovariectomized submitted to fructose overload (RHOF) and combined trained hypertensive ovariectomized submitted to fructose overload (CHOF). Arterial pressure (AP) signals were directly recorded. Vascular autonomic modulation was evaluated by spectral analysis. The cardiac oxidative stress was evaluated by lipoperoxidation (LPO) determination. The association of fructose overload and hormone deprivation promoted an increase in AP (HOF: 174±4 vs. H: 146±4 mmHg), heart rate (HOF: 393±10 vs. H: 352±13 bpm), VAR-SAP (HOF: 77.8±11.9 vs H: 31.1±2.6 mmHg²), LF-SAP (HOF: 10.6±2.3 vs H: 5.0±0.9 mmHg²) and LPO, and reduced baroreflex sensitivity (tachycardia response: HOF: 1.06±0.06 vs. H: 1.91±0.17 bpm/mmHg). All exercise training protocols were able to reduce LPO and LF-SAP. It was noted that only the combined exercise training was able in reducing AP (CHOF: 158±4 mm Hg) and heart rate (CHOF: 303±5 bpm). The AP reduction noted only in the CHOF group may be associated with an improve in baroreflex sensitivity, represented by an increase of tachycardic response observed only in the CHOF (1.62±0.1 bpm/mmHg) and in the AHOF (1.54 ±0.07 bpm/mmHg) groups and a reduction of
VAR-PAS observed only in the CHOF (30.31±3.85 mmHg²) and in the RHOF (31±2.65 mmHg²) groups. In conclusion, fructose overload induced impairment in hemodynamic, vascular autonomic control and increased oxidative stress in hypertensive rats submitted to ovarian hormones deprivation. However, all exercise training protocols showed a beneficial role. Moreover, the combined exercise training showed additional improvement, suggesting that this could be a better approach than isolated aerobic and resistance training.


Funding: No
Funding Component: P168

20-HETE-mediated Neutrophil Adhesion Impairs Coronary Collateral Growth in Metabolic Syndrome

**Gregory Joseph**, Amanda Soler, Rebecca Hutcheson, Ian Hunter, Brenda Hutcheson, New York Medical Coll, Valhalla, NY; Chastity Bradford, Tuskegee Univ, Tuskegee, AL; Katherine H Gotlinger, New York Medical Coll, Valhalla, NY; John R Falck, Univ of Texas Southwestern Medical Ctr, Dallas, TX; Spencer Proctor, Univ of Alberta, Alberta, AB, Canada; Michal L Schwartzman, Petra Rocic, New York Medical Coll, Valhalla, NY

Transient, repetitive myocardial ischemia (RI)-induced coronary collateral growth (CCG) is impaired in metabolic syndrome patients and animal models. Endothelial cell (EC) dysfunction and chronic inflammation are hallmarks of metabolic syndrome. We showed that while in normal animals (SD), RI induces transient infiltration of monocytes, associated with successful CCG, in metabolic syndrome rats (JCR), RI induces sustained accumulation of neutrophils, which contributes to compromised CCG. 20-hydroxyicosatetraenoic acid (20-HETE) is a pro-inflammatory metabolite of arachidonic acid. Its role in the regulation of CCG is unknown. We hypothesized that enhanced 20-HETE-mediated neutrophil adhesion to ECs and consequent EC dysfunction and apoptosis result in impaired CCG in metabolic syndrome. P-selectin and ICAM-1 expression was increased ~40% in JCR vs. SD rats. This increase was prevented by 20-HETE antagonists, 20-SOLA or 20-HEDGE. 20-HETE antagonists also prevented neutrophil accumulation observed in JCR rats. Coronary arteries from JCR rats exhibited reduced endothelium (Ach)-dependent vasodilation (20% JCR vs. 50% of max. SD). RI-induced eNOS activation and NO production were likewise decreased (~60% and ~70%, respectively) in JCR vs. SD rats. EC apoptosis (TUNEL) was severely increased in response to RI in JCR rats (~75% vs. SD). Neutrophil adhesion-blocking antibodies partially attenuated EC apoptosis (~70%) and EC dysfunction (~75% eNOS activation and NO production, 75% Ach-dependent vasodilation). 20-HETE antagonists fully reversed impaired endothelium-dependent vasodilation, eNOS activation, NO production and prevented EC apoptosis. Finally, impaired CCG in JCR rats (collateral-dependent blood flow, microspheres) was completely restored by 20-HETE antagonists (CZ/NZ flow was 0.76±0.07 in JCR+20-SOLA, 0.84±0.05 in JCR+20-HEDGE vs. 0.11±0.02 in JCR vs. 0.84±0.03 ml/min/g in SD rats) and partially restored by neutrophil-blocking antibodies (0.49±0.05 ml/min/g). Taken together, these results indicate that 20-HETE-dependent neutrophil adhesion and accumulation compromises EC survival and function leading to impaired CCG. 20-HETE antagonists could provide therapy for restoration of CCG in metabolic syndrome.

G. Joseph: None. A. Soler: None. R. Hutcheson: None. I. Hunter: None. B. Hutcheson: None. C.
Dipeptidyl Peptidase-4 Regulates Hematopoietic Stem Cell Activation in Response to Chronic Stress

Xian Wu Cheng, Nagoya Univ Sch Med, Nagoya, Japan; Enbo Zhu, Yanbian Univ Hosp, Yanji, China; Lina Hu, Yanna Lei, Limei Piao, Aiko Inoue, Masafumi Kuzuya, Nagoya Univ Sch Med, Nagoya, Japan

Background: Dipeptidyl peptidase-4 (DPP4) inhibition exhibits multiple pleotrophic effects. Hematopoietic stem cell (HSC) activation has been implicated in the pathogenesis of stress-related metabolic disorder and cardiovascular disease. Given that interaction between β3-adrenergic receptor (Adrβ3) signaling and the immune system may link stress and the initiation and progression of disorders, we investigated whether DPP4 regulates immune over-reactions in a chronic stress mouse model, focusing on HSC activation.

Methods and Results: Male 8-week-old mice fed a normal diet underwent chronic stress were randomly assigned to one of three groups and administered vehicle or a low or high dose of the DPP4 inhibitor anagliptin. Control mice were left undisturbed. The stress increased the blood and brain DPP4 activity, the levels of plasma adrenaline and noradrenaline, and the bone marrow (BM) niche cell adrenergic receptor (Adrβ3) expression, and it decreased the levels of plasma glucagon-like peptide (GLP-1) as well as brain GLP-1 receptor (GLP-1R) and BM Cxcl12 expressions. These changes were reversed by DPP4 inhibition. The stress activated the BM sca-1highc-KithighCD48lowCD150high HSC proliferation, giving rise to high levels of blood leukocytes and monocytes. These DPP4 inhibition-related benefits were mimicked by DPP4 depletion and by GLP-1R activation. Adrβ3 inhibition mitigated BM Cxcl12 expressions and HSC activation.

Conclusions: DPP4 activity appears to regulate chronic stress-induced BM HSC activation and inflammatory cell production via an Adrβ3-CXCL12-dependent mechanism that is mediated by the GLP-1-GLP-1R axis, suggesting that the inhibition of DPP4 or the stimulation of GLP-1R may have applications in the treatment of inflammatory diseases.


Funding: No

Funding Component: P169

Tonin Overexpression in Mice Diminishes Sympathetic Autonomic Modulation and Suppresses Adrenergic Response

Zaira Palomino Jara, Federal Univ of São Paulo, São Paulo, Brazil; Ivana C. Moraes-Silva, Fernando Santos, Heart Inst, Univ of São Paulo, São Paulo, Brazil; Leandro E. Souza, Heart Inst, Univ of São Paulo, São Paulo, Brazil; Maria Claudia Irigoyen, Dulce Elena Casarini, Heart Inst, Univ of São Paulo, São Paulo, Brazil

Background: Increased blood pressure (BP) and decreased heart rate (HR) variabilities are associated with higher risk of cardiovascular morbidity. It has been reported an increase of serine-proteases of the renin-angiotensin system in cardiovascular pathophysiology, and an important serine present in the brain, heart and vasculature is the tonin enzyme. Aim: Evaluation of cardiovascular autonomic profile in transgenic tonin mice (TGM´rton)) and the impact of AT1 receptor blocker, losartan.

Methods: Male C57BL/6 (Wt) and
TGM´(rton)mice were canulated for BP signals registering (Windaq, 4MHz) for 30 min in baseline and more 30 min after losartan injection (1mg/kg). BP and HR variabilities were analyzed in frequency domain by FFT method. Low frequency (LF) and high frequency (HF) components were identified for sympathetic and parasympathetic modulations analysis. Catecholamines (CA) were quantified by HPLC method. Results: TGM´(rton) presented higher BP (136 ± 3 vs 120 ± 3 mmHg, p =0.007), lower cardiac LF (33 ± 2.4 vs 66 ± 3.3 nu, p = 0.0004), higher HF(67 ± 2.4 vs 34 ± 3.4 nu, p = 0.0006), lower LF/HF (0.64 ± 0.06 vs 2.8 ± 0.11), and lower alpha index (0.35 ± 0.05 vs 1.35 ±0.13 ms/mmHg²) than Wt. After losartan injection, BP and LF of systolic BP were decreased in Wt mice in relation to its baseline (133 ± 3.8 vs 124 3.4 mmHg, p=0.0005; 66 ± 3.3 vs 56 ±5.8 mmHg², p=0.0004; respectively). Conversely, TGM´(rton) group presented an additional decrease in cardiac LF (32.7 ±2.4 vs 8 ±0.6, p=0.0004), and increase in HF (67 ± 2.4 vs 91 ± 0.6 nu, p= 0.0009) in relation to its baseline and Wt after losartan. Additionally, losartan in TGM´(rton) mice caused a decrease in BP (140 ±2.6 vs 121 ± 1.6mmHg, p=0.0005) with no alteration in LF of systolic BP (9.9 ± 0.6 vs 9.6 ± 0.6 mmHg² ). CA to sympathetic modulation ratio was 9x higher in TGM´(rton) than Wt at baseline. As we no response in LF of systolic BP despite CA increase after losartan in TGM´(rton), this augment in CA/LF ratio indicates that adrenergic receptors are desensitized in TGM´(rton) mice. Conclusion: Activation of adrenergic receptors play a key role in the sympathetic modulation to the vessels rather than to the heart in the TGM´(rton) mice, in which activation of AT1 receptors seem to be more relevant.


Funding: No
Funding Component: P171

Superoxide via Transcription Factor Sp3 Up-regulates Renal Angiotensin II Receptor Function

Mohammad Saleem, Univ of Houston, Houston, TX

Oxidative stress is linked to the up-regulation of angiotensin II type 1 receptor (AT1R) function and hypertension. Here we tested the mechanism of oxidative stress-associated up-regulation of AT1 receptor function in human kidney HK2 cells. Diethylthiocarbamate (DETC), a superoxide dismutase inhibitor, and hydrogen peroxide (H2O2) increased DHE and DCFHDA fluorescence respectively [DHE fluorescence (Control vs DETC vs DETC + tempol vs H2O2: 0.5397 ± 0.07057, 1.463 ± 0.1671, 0.2661 ± 0.01776, 0.6406 ± 0.04821); DCFH fluorescence (Control vs H2O2 vs 3-AT vs DETC: 555.9 ± 21.22, 673.5 ± 37.05, 736.1 ± 33.79, 427.7 ± 22.17)]. DETC increased nuclear levels of Sp3 protein that were attenuated with tempol (Control vs DETC, vs DETC + tempol: Sp3, 0.50 ± 0.08, 0.68 ± 0.14, 1.04 ± 0.30 densities). However, H2O2 had no significant effect on nuclear Sp3 (Control vs H2O2, vs H2O2 + tempol: Sp3, 0.50 ± 0.08, 1.28 ± 0.21, 0.52 ± 0.12 densities). In transfection studies, Sp3 plasmid increased (Control vs Sp3: 0.1165 ± 0.01, 0.3810 ± 0.03, densities) while Sp3 siRNA decreased (Control siRNA vs Sp3 siRNA: 1.11 ± 0.25, 0.64 ± 0.06, densities) the levels of AT1R protein in cell lysate. DETC increased AT1R protein expression that was attenuated by tempol, measured by immunoblotting (Control vs DETC vs DETC + tempol: 0.50 ± 0.08, 1.28 ± 0.21, 0.52 ± 0.12 densities). Furthermore, DETC increased cell membrane levels of AT1R protein as measured by biotinylation and immunoblotting. This effect was attenuated by tempol. (Control
vs DETC vs DETC + tempol: 100 ± 0.0, 189.8 ± 0.34.60, 102.3 ± 10.77 densities, percent change). Moreover, DETC increased PKC activity in response to angiotensin II, an indicator of AT1R function. This effect was attenuated with candesartan and tempol. (Control vs Ang II vs DETC + AngII vs DETC + Cand + AngII vs DETC + tempol: 100.0 ± 0.0, 162.0 ± 12.97, 224.9 ± 52.97, 126.0 ± 6.133, 73.32 ± 35.01 percent change) These results suggest that superoxide but not H2O2 increase nuclear Sp3 protein that up-regulates renal AT1R expression and function.

M. Saleem: None.

Funding: No

Funding Component: P172

Activation of Pgc1α by EET Stimulates Insulin Sensitivity, Normalizes Blood Pressure and Increases Mitochondrial Oxphos in Obese Mice

Shailendra P Singh, New York Medical Coll, valhalla New York, NY; Maayan Waldman, Joseph Schragenheim, Lars Bellner, New York Medical Coll, Valhalla, NY; Jian Cao, First Geriatric Cardiology Div, Chinese PLA General Hospital, China; Michael Arad, Leviev Heart Ctr Sheba Medical Ctr, Tel Aviv University, Israel, Israel; Edith Hochhauser, Felsenstein Medical Res Inst, Cardiac Research Laboratory, Israel; Nader Abraham, New York Medical Coll, Valhalla, NY

Background/Objectives: Obesity is a risk factor in the development of type 2 diabetes mellitus (DM2), which is associated with increased morbidity and mortality, predominantly as a result of cardiovascular complications. Increased adiposity is a systemic condition characterized by increased oxidative stress (ROS), inflammation, inhibition of anti-oxidant genes such as HO-1 and increased degradation of epoxyeicosatrienoic acids (EETs). Hypothesis

We postulate that EETs increase peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) activity, which controls mitochondrial function, oxidative metabolism and may also increase antioxidants and HO-1 gene expression. Methods: C57/B16 mice were fed a high fat (HF) diet for 26 wks. The protocol comprised three groups: A) WT, B) HF control and C) HF-treated with EET agonist (EET-A). Renal and visceral fat tissues were harvested to measure signaling protein. Consumption was measured at 6 and 24 wks. Mice were used to assess insulin levels, insulin sensitivity, blood pressure and mitochondrial OXPHOS and mitochondrial biogenesis (Mfn1, 2 and Opal), and oxygen consumption (VO2). Results: Animals on a HF diet exhibited increased body weight, fat content, fasting blood glucose levels, systolic blood pressure (BP) and a significant reduction in VO2. Administration of EET-A to HF-fed mice decreased the RQ (VCO2/VO2) ratio and normalized BP. The HF diet produced increased levels of the adipogenic markers MEST, aP2, C/EBPα and FAS. EET-A attenuated these perturbations through an increase in renal and adipose tissue PGC1α levels. The EET-mediated HO-1 induction increased mitochondrial function as measured by OXPHOS, MnSOD and thermogenic genes, TFAM, UCP1 and SIRT 1. EET-A also increased adiponectin levels, and insulin receptor phosphorylation IRP Tyr 972 and 1146 and normalized glucose levels. Conclusion: These data show that an EET agonist increased PGC-1α-HO-1 levels thereby providing metabolic protection and increased VO2 consumption in HF-induced obesity in mice. This novel finding suggests that the EET-mediated PGC-1α activation is essential to increase HO-1 levels, mitochondrial biogenesis, and to decrease mitochondrial ROS and adiposity.

S.P. Singh: None. M. Waldman: None. J. Schragenheim: None. L. Bellner: None. J. Cao:
Caloric Restriction Attenuates Diabetic Cardiomyopathy Through the Recruitment of the Antioxidant System

Maayan Waldman, Cardiac Res Lab, Felsenstein Medical Res Inst, Leviev Heart Ctr, Sheba Medical Ctr, Tel Hashomer and Sackler Sch of Med, Tel-Aviv Univ, Petach-Tikva, Israel; Keren Cohen, Cardiac Res Lab, Felsenstein Medical Res Inst, Leviev Heart Ctr, Sheba Medical Ctr, Tel Hashomer and Sackler Sch of Med, Tel Aviv Univ, Petach-Tikva, Israel; Michael Arad, Leviev Heart Ctr, Sheba Medical Ctr, Tel Hashomer and Sackler Sch of Med, Tel Aviv Univ, Petach-Tikva, Israel; Nader G. Abraham, Dept Med and Pharmacology, New York Medical Coll, Valhalla, NY; Michal Laniado-Schwartzman, Dept Pharmacology, New York Medical Coll, Valhalla, NY; Danny Gurfield, Dan Aravot, Edith Hochhauser, Cardiac Res Lab, Felsenstein Medical Res Inst, Tel-Aviv Univ, Petach-Tikva, Israel

Introduction: Insulin resistance negatively impacts the diabetic heart in various ways, that include impaired insulin-mediated glucose uptake and a reduction in intracellular signalling. Diabetic cardiomyopathy is independent of coronary artery disease and is characterized by increased oxidative stress and extensive fibrotic changes, leading to increased myocardial stiffness and the development of diastolic dysfunction. Caloric restriction (CR) is cardioprotective mainly through its catabolic activity and increased insulin sensitivity. We examined the effect of CR on the development of diabetic cardiomyopathy and changes in oxidative stress and antioxidant genes.

Methods: Leptin resistant (db/db) mice suffer from obesity and diabetes. Mice were treated for 4 weeks with angiotensin II (AT) to induce severe cardiomyopathy. Mice under CR were fed 90% of their normal food intake for 2 weeks and 65% for an additional 2 weeks. Each group consisted of 5-6 animals.

Results: CR attenuated obesity and the cardiomyopathy phenotype in diabetic mice. CR reduced body weight and heart weight in diabetic mice when compared to control animals (33.7±7.9g vs. 44 ±5.9g; 0.137±0.023g vs. 0.17±0.02g respectively, p<0.05); and lowered blood glucose (576±167mg/dL vs 702.5±309 mg/dL, p<0.05). Echocardiography indicated that CR attenuated the hypertrophic phenotype in the diabetic mice when compared to control animals (LV internal diameter 3.34±0.46mm vs. 4.06±0.36mm, p<0.01). Diabetic mice treated with AT suffer from oxidative stress as evident in a 110% increase in serum MDA levels (p<0.011), a reduction of 81% in adiponectin (p<0.001) and 65% in PGC-1α (p<0.0046) mRNA levels in cardiac tissue of diabetic mice compared to WT mice. The attenuation of diabetic cardiomyopathy after CR was accompanied by a reduction in serum MDA levels (p<0.028) and an increase in cardiac adiponectin, HO-1 and PGC-1α levels (p<0.05).

Conclusion: These results indicate that a short term CR attenuated the development of AT induced diabetic cardiomyopathy through the activation of the adiponectin- PGC-1α- HO-1-axis. This appears to be a critical module in protecting the diabetic heart from the development of cardiomyopathy.


Funding: No
Funding Component: P174
17-hydroxyprogesterone Caproate Improves Blood Pressures and Placental Cytolytic Nk Cells in Response to Placental Ischemia During Pregnancy

Lorena M Amaral, Jamil Elfarra, Denise C Cornelius, Mark W Cunningham Jr, Tarek Ibrahim, Babbette LaMarco, The Univ of Mississippi Medical Ctr, Jackson, MS

Preeclampsia (PE), new onset hypertension, is characterized by decreased fetal weight, elevated cytolytic natural killer (NK) cells and placental ischemia during pregnancy. Cytolytic NK are thought to play a role in fetal demise as they have also been shown to be increased in patients suffering from miscarriage. Currently, there is no effective treatment for PE except for early delivery, making PE the leading cause for premature births worldwide. Multiple injections of 17-hydroxyprogesterone caproate (17-OHPC) is used for prevention of preterm labor, but not for management of PE. We have shown that injections of 17-OHPC to the RUPP rat model of PE improves some but not all facets of PE observed in this model. Therefore this study was designed to test the hypothesis that injections of 17-OHPC on both day 15 (GD15) and GD (18) improve outcomes of hypertension in response to placental ischemia. To do so, 17-OHPC (3.32mg/kg) was administered intraperitoneally on GD 15 and 18 to reduced uterine perfusion pressure (RUPP) rats, carotid catheters were inserted on GD 18 and blood pressure (MAP) and placental cytolytic NK cells were measured on GD 19. MAP in normal pregnant (NP) rats (n=8) was 104±4,119±5 in RUPP rats (n=5) and 102±5 mmHgin RUPP+17-OHPC GD15 &18 (n=4), p <0.05. Total number of placental NK cells was 8.5±3 in NP, 20±2 in RUPP rats, which decreased to 4.7±3 % in RUPP+17-OHPC GD15 &18, p<0.05. Activated placental NK cells was 3.4±1.6 in NP, 10.5±2.3 in RUPP, which improved to 2.7±2.7 % in conclusion, administration of 17-OHPC on days 15 and 18 decreased hypertension and NK cells that are associated with PE in the RUPP rats and should be considered for addition to the management of PE.


Funding: No

Funding Component: P175

Soluble Guanylate Cyclase Activator Attenuates Tumor Necrosis Factor-α Induced Production of Endothelin-1 from Human Glomerular Endothelial Cells

Bhavisha Bakrania, Frank T. Spradley, Univ of Mississippi Medical Ctr, Jackson, MS; Simon Satchell, Univ of Bristol, Bristol, United Kingdom; Joey P. Granger, Univ of Mississippi Medical Ctr, Jackson, MS

Preeclampsia (PE) is a disorder associated with maternal hypertension, endothelial dysfunction and reductions in renal hemodynamics. Placental ischemia leads to increases in circulating maternal anti-angiogenic and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) that induce endothelin-1 (ET-1), a potent vasoconstrictor. PE is also associated with depletion of nitric oxide, a facilitator of vasodilation, which binds to soluble guanylate cyclase (sGC), and synthesizes cGMP. In addition to promoting vasodilation, sGC activators and stimulators inhibit smooth muscle proliferation, leukocyte recruitment and platelet aggregation and are therefore, currently in clinical trials for treating cardiopulmonary disease. Although it is known that activating the nitric oxide signalling pathway induces vasodilation, its ability to inhibit TNF-α induced renal glomerular
endothelial ET-1 production is unknown. We tested the hypothesis that cinaciguat, a sGC activator, attenuates ET-1 production induced by TNF-α in conditionally immortalized human glomerular endothelial cells. Cells were cultured; starved for 48 h; and treated for 12 h resulting in the following 4 groups having N=6/group: 1) Untreated, 2) 10 ng TNF-α 3) 10 µM cinaciguat + 10 ng TNF-α, and 4) 20 µM cinaciguat + 10 ng TNF-α. TNF-α (10 ng, 67.25±3.2 pg/mL) significantly increased ET-1 production compared to the untreated group (43.6±4.3 pg/mL, P<0.01). Interestingly, both cinaciguat treatment groups attenuated TNF-α induced ET-1 production, with significant reductions at a higher dose (20 µM, 57.38±1.42 pg/mL, P=0.02; 10 µM, 58.6±2.32 pg/mL, P=0.07). The results of this study demonstrate that activating sGC can attenuate ET-1 production. In conclusion, these findings suggest there is a therapeutic potential for treating preeclampsia with sGC activators.

B. Bakrania: None. F.T. Spradley: None. S. Satchell: None. J.P. Granger: None.

Funding: No

Maternal and Neonatal Outcomes in Normal and Preeclamptic Pregnancies - A Comparative Prospective Study

Ram R Kalagiri, Scott & White Healthcare/TAMHSC, Temple, TX; Syeda H Afroze, Texas A&M Health Science Ctr Coll of Med, Temple, TX; Niraj Vora, Nathan Drever, Madhava R Beeram, Thomas J Kuehl, Scott & White Healthcare/TAMHSC, Temple, TX; David C Zawieja, Texas A&M Health Science Ctr Coll of Med, Temple, TX; Mohammad N Uddin, Scott & White Healthcare/TAMHSC, Temple, TX

Background: Preeclampsia (PreE) is de novo development of hypertension and proteinuria after 20 weeks of gestation with multiple pathophysiologic triggers affecting 3-8% of pregnancies. PreE has a significant link to alterations of feto-placental stress that pass to the offspring causing detrimental effects. We assessed and compared the pregnancy outcomes between patients with and without PreE. Methods: We recruited 35 normal pregnant (NP) and 25 PreE consenting patients in an IRB approved prospective study at Scott & White Memorial Hospital, Temple, Texas. We evaluated maternal age, body mass index (BMI), blood pressures, proteinuria, and gestational age at delivery. We divided the PreE subjects into early (before 34 weeks) and late PreE (after 34 weeks) groups and compared their outcomes. Placental thickness and volumes were measured. We also evaluated neonates for intrauterine growth restriction (IUGR), gestational age at birth, anthropometric measurements including Ponderal Index (PI), length of hospitalization and other neonatal complications. Comparisons were performed using Student’s t test. Results: Maternal: The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were higher in PreE (SBP 166 ± 11; DBP 93 ± 10) compared to normal pregnancies (SBP 122 ± 10; DBP 74 ± 9; p <0.05 for each case). PreE mothers had higher urinary protein excretion (457mg/24h ± 140) compared to NP (160 mg/24h ± 44; p <0.05 for each case). We did not find any difference in body mass index (BMI). Placenta: The placental thickness in early PreE subjects was 25mm compared to 32mm in late PreE (p <0.05) and placental volume in early PreE 296 cm³ compared to NP 393cm³ (p <0.05). Neonatal: The average GA at delivery was lower in PreE (34.8 wks. ± 4) compared to NP (39.2 weeks ± 0.3; p <0.05). Average hospital stay for PreE babies were longer (20 days ± 5) compared to NP (2 days ± 1; p <0.05). The PreE babies were SGA with lower PI (2.28 ± 0.3) compare to the NP babies (2.95 ± 0.2; p <0.05). Gestational age at delivery in early PreE is 32.4 weeks vs 36.8 weeks in late
PreE (p <0.05). About 56% of the infants who are born to early PreE are small for gestational age (SGA) and 30% of the infants who are born to late PreE are SGA. PreE babies had multiple complications compared to NP babies.


Funding: No
Funding Component: P177

**Reduced Expression of Regulator of G-Protein Signaling-2 (RGS2) in the Placenta During Preeclampsia**

Katherine J Perschbacher, Donna A Santillan, Eric J Devor, Sabrina M Scroggins, Jeremy A Sandgren, Gary L Pierce, Mark K Santillan, Rory A Fisher, Katherine N Gibson-Corley, Justin L Grobe, Univ of Iowa, Iowa City, IA

Preeclampsia (PE) is a serious cardiovascular condition of late pregnancy. Genetic risk factors and the early-gestational etiology remain largely unclear, though evidence supports excessive activation of Gαq signaling within the placenta in response to various hormones including vasopressin, endothelin, and angiotensin. Regulator of G-protein Signaling 2 (RGS2) acts as an endogenous terminator of Gαq signaling, and previous association studies have identified an increased risk for PE and its sequelae in women carrying a single nucleotide polymorphism that is expected to reduce levels of RGS2. We hypothesized that RGS2 is expressed in placental trophoblasts, and that reduced expression of RGS2 in placental tissue may represent a risk factor for the development of PE. Whole placenta samples and clinical data from preeclamptic and clinically-matched control pregnancies were obtained from the University of Iowa Maternal-Fetal Tissue Bank (IRB#200910784) and examined for mRNA levels of the B/R4 family of RGS proteins, including RGS2. Of the members examined (RGS2, -3, -4.2, -4.3, -4.4, -4.5, and -5) in control placentas (n=9), only RGS2 (Ct 28.8±0.7 vs 18S Ct 12.1±0.4) and RGS4.3 (Ct 23.0±0.4 vs 18S Ct 13.3±0.3) transcripts were expressed above background levels. RGS2 protein expression was then confirmed in human placental tissues by Western blot. RGS2 mRNA expression was 3-fold higher in fetal (amniotic, p<0.05) layers than maternal (decidual) layers. In preeclamptic placenta (n=11), RGS2 may be suppressed (1.0±0.4 vs 0.2±0.3-fold, p=0.1) while RGS4.3 remains unchanged (1.0±0.4 vs 1.1±0.4 fold, p=0.8). Initial immunohistochemical detection confirms cytoplasmic localization of RGS2 in trophoblasts of wildtype mouse placenta, despite exclusive nuclear localization in other tissues. We conclude that human placenta expresses RGS2, and that this expression may be suppressed during preeclampsia. Loss of RGS2 expression may result in disinhibited trophoblast Gαq signaling, and ultimately placental insufficiency.

K.J. Perschbacher: None. D.A. Santillan: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. E.J. Devor: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. S.M. Scroggins: None. J.A. Sandgren: None. G.L. Pierce: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. M.K. Santillan: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. R.A. Fisher: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA.
Vasopressin Induces Discrete Symptoms of Preeclampsia Through Receptor- and Gestational Age-specific Mechanisms

Jeremy A Sandgren, Danny W Linggonegoro, Nicole A Pearson, Katherine J Perschbacher, Donna A Santillan, Sabrina M Scroggins, Katherine N Gibson-Corley, Gary L Pierce, Mark K Santillan, Justin L Grobe, Univ of Iowa, Iowa City, IA

Preeclampsia (PE) is a common late-gestational disorder characterized by de novo pregnancy specific hypertension, proteinuria, and renal glomerular endotheliosis (RGE). Arginine vasopressin (AVP) secretion (as measured by copeptin) is elevated as early as 6 weeks gestation in pregnancies which later develop PE, and chronic low-dose AVP infusion is sufficient to phenocopy PE in pregnant mice. However, the identity and timeframe of involvement of specific AVP receptors initiating discrete symptoms of PE in this model remain unclear. Wildtype C57BL/6J mice were instrumented with subcutaneous osmotic minipumps to infuse AVP (24 ng/hr) and/or inhibitors during gestation. To clarify the involvement of AVP V₁A and V₂ receptors, one cohort of saline- or AVP-infused pregnant mice was simultaneously infused with the combined V₁A+V₂ antagonist, conivaptan (22 ng/hr). Conivaptan co-treatment ameliorated the hypertension phenotype (SBP on gestational day (GD)15: saline 108.3±2.1, n=24; AVP 120.5±2.1, n=17; conivaptan 112.8±4.1, n=8; AVP+conivaptan 109.8±3.5 mmHg, n=11) but did not prevent proteinuria (on GD17: saline 46.4±6.4, n=15; AVP 79.3±8.5, n=15; conivaptan 64.6±6.7, n=7; AVP+conivaptan 72.9±12.3 mg/mL, n=11) or RGE. To clarify the important timeframes of involvement of AVP for discrete symptoms, subsets of mice were infused with AVP throughout gestation or to only GD10. Continuous infusion of AVP was required to maintain the hypertensive phenotype, as infusion to only GD10 initially elevated blood pressure (SBP at GD10: saline 103.7±1.3, n=26; AVP to GD10 111.5±1.6 mmHg, n=19) but failed to sustain hypertension for the remainder of gestation (SBP at GD15: 112.2±1.3 mmHg, n=19). In contrast, infusion of AVP only to GD10 caused a sustained proteinuria (at GD17: 88.2±9.2 mg/mL, n=19). Infusion of AVP only to GD3 had no effect on any examined endpoint. These data support a specific role for AVP V₁A/V₂ receptors throughout gestation for the hypertension but not proteinuria or RGE phenotypes of PE. Our results therefore indirectly support an early-gestational role for other receptors (perhaps V₁B, cullin-5, or the oxytocin receptor) in the proteinuria and RGE phenotypes of this disorder.

J.A. Sandgren: None. D.W. Linggonegoro: None. N.A. Pearson: None. K.J. Perschbacher: None. D.A. Santillan: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. S.M. Scroggins: None. K.N. Gibson-Corley: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. G.L. Pierce: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. K.N. Gibson-Corley: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA.
already received); Significant; NIH, AHA. **M.K. Santillan**: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. **J.L. Grobe**: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA.

**Funding**: Yes
**Funding Component**: National Center
**P179**

**Neonatal Outcomes in Normal and Preeclamptic Pregnancies: A retrospective Comparative Study**

**Niraj Vora**, Ram R Kalagiri, Venkata N Raju, Nathan Drever, Madhava R Beeram, Lea Mallett, Mohammad N Uddin, Scott & White Healthcare/TAMHSC, Temple, TX

**Background**: Preeclampsia (PreE), a de novo development of Hypertension in consort with proteinuria after 20 weeks of gestation is the leading cause of morbidity and mortality in mother and the offspring. It affects approximately 3-8% of overall pregnancies. Although, specific etiologies remain unknown, it has been supported by various studies that PreE is not just a single disorder, but a syndrome of pertinent multiple pathophysiological factors.

**Methods**: An IRB approved retrospective chart review over a year (January 2014 to December 2014) was conducted of all pregnancies occurred at Baylor Scott and White Health System, Temple, Texas (N = 3704). We divided all pregnancies into two separate groups: PreE (N = 299) vs. Non PreE (N = 3405). We compared the neonatal outcomes between two groups including their offspring’s gestational age, birth weight, admission rate to Neonatal Intensive Care Unit (NICU), occurrence of bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), hypoglycemia, thrombocytopenia, intraventricular hemorrhage (IVH) and length of hospital stay (LOS).

**Results**: We found amongst these two groups, infants born to PreE mothers have significantly lower birthweight (Mean = 2807 grams, SD = 841 grams) compared to Non PreE mothers (Mean = 3383 grams, SD = 619 grams) (P<0.05), significantly lower GA (Mean = 36.7 weeks, SD = 3.25 weeks) compared to Non PreE group (Mean = 38.7 weeks, SD = 2.1 weeks) (P<0.05), significantly higher rate of BPD (11%) compared to Non PreE group (6.9%)(P<0.05), significantly higher occurrence of hypoglycemia (26%) compared to non PreE group (20%) (P<0.05), significantly higher rate of thrombocytopenia (28%) compared to Non PreE group (17%) (P<0.05) and significantly higher length of hospital stay (Mean = 19 days, SD = 20 days) compared to Non PreE group (Mean = 14 days, SD = 20 days) (P<0.05).

**Conclusion**: We can conclude from this retrospective analysis that infants born to PreE mothers have lower birth weight indicating the intrauterine growth restriction and the lower gestational age indicating preterm birth. Moreover, the data indicate the higher rate of BPD, hypoglycemia, thrombocytopenia and requirement of increased length of hospital stay in infants born to PreE mothers compared to Non PreE mothers.


**Funding**: No
**Funding Component**: P180

**Quantification of Substance P in Human Blood by Mass Spectroscopy**

**Scott Hubers**, Shouzuo Wei, Nancy Brown, Vanderbilt Univ Medical Ctr, Nashville, TN
**Background:** Substance P (SP), a tachykinin, may contribute to the effects of ACE inhibitors. Measurement of SP has been limited by its short half-life and cross-reactivity with metabolites. Here we quantify SP in human blood using mass spectroscopy (MS). **Methods:** Venous blood was obtained from healthy subjects before and during arterial infusion of SP (2, 4, and 8 pmol/min). Venous blood (3 mL) was immediately added to 9 mL chilled ethanol to denature proteases. After 30 min at 4°C, samples were centrifuged and the supernatant stored at -70°C. $^{13}$C$_6$, $^{15}$N$_4$ SP internal standard was then added to the ethanolic supernatant. Samples were purified with Nexus and C$_{18}$ Sep-Pak cartridges. Sample was further resolved by UPLC (40°C) on a C18 column using solvent mixture of 5% acetonitrile/0.1% formic acid at a flow rate 0.14 mL/min to 30% acetonitrile/0.1% formic acid in 7 min with a linear gradient, and quantified by positive ESI-MS/MS on a “Vantage” triple quadrupole MS with SRM.

**Results:** The lower limit of detection of SP was 0.1 pg. Linearity was confirmed over a 100-fold concentration range. Precision was measured by analyzing five 2-mL aliquots of pooled ethanolic supernatant from 3 volunteers collected during SP 8 pmol/min. The coefficient of variation was 2.2%. The mean SP concentration was 2.3 pg/mL at baseline and increased with intra-arterial dose (Figure).

**Conclusion:** SP can be measured in blood using MS. This assay can be used to study the effects of ACE, DPP4, and neprilysin inhibitors on SP.
in vitro. Based on this finding, however, a very bold hypothesis is formed to test that abnormal cilia function results in vascular hypertension. Though both primary cilia and muscarinic acetylcholine receptors play important roles in vascular hypertension, their relationship has never been explored. To determine the roles of the cholinergic system and mechanosensory cilia, we studied the effects of acetylcholine on ciliary length and function in wild-type (WT) and mechanosensitive cilia mutant endothelial cells (Pkd1−/− and Tg737opk/opk). We show for the first time that mouse vascular endothelia exhibit muscarinic receptor-type 1, 3 and 5 (AChM1, 3, and 5), which co-localizes to primary endothelial cilia. AChM3R activation significantly increases cilia length in cells treated with AChM3R agonist compared to non-treated cells (1.63±0.01 vs.1.92±0.01).

Furthermore, the chemosensory function of cilia can alter the mechanosensory function through changes in sensitivity to fluid-shear stress. We propose that activated ciliary AChM3R has a functional mechanosensory role in endothelial cells. A series of conditional mouse models are used, coupled with high-resolution microscopy techniques. We used vascular-specific mouse models of Pkd1, Tg737 and AChM3R to study systolic/diastolic blood pressure. Our data corroborate our hypothesis, that cilia function knockout in the vascular system is associated with elevated blood pressure compared to wildtype controls (123±6 vs. 135±6 systolic and 79±6 vs. 89±5 diastolic).

W. AbouAlaiwi: None. H.C. Saternos: None.

Funding: No

Funding Component: P182

The Novel Perivascular Adipose Tissue Adipokine, Chemerin, Signals Through Gi- and Calcium-Dependent Mechanisms

David J Ferland, Emma S Darios, Richard R Neubig, Benita Sjögren, Nguyen Truong, Rosa Torres, Thomas S Dexheimer, Janice Thompson, Stephanie W Watts, Michigan State Univ, East Lansing, MI

Chemerin is an adipokine associated with inflammation, increased blood pressure, and may be a link between the pathologies of obesity and hypertension. We tested the hypothesis that chemerin-induced contraction of the vasculature occurs via the chemerin receptor and calcium flux in smooth muscle cells. Known mediators of the amplified arterial responsiveness seen in hypertension (L-type Ca2+ channels, Src, and Rho kinase) were interrogated by isometric contraction of rat aortic rings in parallel with calcium kinetics of rat aortic smooth muscle cells. Western blots were also used to observe phosphorylation of Erk/MAPK. Chemerin-9 (nonapeptide of the chemerin S157 isoform) caused a concentration-dependent contraction of isolated aorta (EC50 100 nM) and elicited a concentration-dependent intracellular calcium response (EC50 10 nM). Both calcium influx and isometric contraction, respectively, were reduced (units of “% of vehicle response”) by Pertussis toxin (Gi inhibitor; 0±3% and 23±9%), verapamil (L-type Ca2+ channel inhibitor; 38±20% and 23±4%), PP1 (Src inhibitor; 43±23% and 15±4%), and Y27632 (Rho Kinase inhibitor; 58±23% and 22±4%) but U73122 (PLC inhibitor) had little to no effect (71±31% and 71±12%). PD098059 (Erk/MAPK inhibitor) did not inhibit chemerin-9 induced contraction (117±19%) and phosphorylation did not change after chemerin-9 stimulation [1.12±0.14 (44 kDa) and 1.11±0.29 (42 kDa) fold-increase with chemerin-9 contraction compared to vehicle, p>0.05]. The chemerin receptor-selective antagonist CCX832 inhibited chemerin-9-induced calcium flux and aortic contraction and calcium flux (0.1±10.3% and 10±7%). These data support a chemerin-
induced contractile mechanism in vascular smooth muscle that functions through the G-linked chemerin receptor to activate L-type Ca2+ channels, Src, and Rho kinase. There is mounting evidence linking chemerin to hypertension and this mechanism brings us one step closer to targeting chemerin as a unique form of therapy.


Funding: No

Funding Component: P183

Chemokine-like Receptor 1 Mediates the Vasoconstrictor Actions of Chemerin in vitro and in vivo

Amanda Kennedy, Peiran Yang, Cai Read, Rhoda Kuc, Janet Maguire, Anthony Davenport, Univ of Cambridge, Cambridge, United Kingdom

Hypertensive patients have significantly higher plasma concentrations of the adipokine chemerin compared with healthy controls, and levels of chemerin positively correlate with systolic and diastolic blood pressure. Chemerin activates chemokine-like receptor 1 (CMKLR1 or ChemR23) but it also activates the ‘orphan’ G protein-coupled receptor 1 (GPR1) which has been linked with hypertension. It is therefore crucial to determine whether one or both of these receptors mediate the constrictor actions of chemerin in the vasculature in order to identify a potential new therapeutic target for the treatment of hypertension. Using immunohistochemistry and molecular biology, we localized chemerin to the endothelium, smooth muscle and adventitia, and CMKLR1 and GPR1 to the smooth muscle in human conduit and resistance vessels. Chemerin activated β-arrestin via heterologously expressed receptors GPR1 (pD2=9.30±0.05) and CMKLR1 (pD2=9.23±0.03) with comparable potency. CCX832, a small molecule antagonist, was fully characterized as highly selective for CMKLR1, with no effect on GPR1 in binding or cell-based functional assays. The C-terminal fragment of chemerin, C9 (chemerin149-157) contracted human saphenous vein (pD2=7.30±0.31) and resistance arteries (pD2=6.23±0.16), and caused a significant increase in blood pressure in rats in vivo (0.2 μmol, 9.1±1.0 mmHg). These actions were blocked by CCX832, confirming for the first time that a single chemerin receptor, CMKLR1, mediates the constrictor response in humans and in vivo. Our data suggest that chemerin activation of CMKLR1 may contribute to elevated blood pressure; this in combination with the known roles of chemerin in metabolic syndrome and diabetes, could lead to increased risk of cardiovascular disease. This study provides proof of principle that the therapeutic potential of selective CMKLR1 antagonists should be explored.

A. Kennedy: None. P. Yang: None. C. Read: None. R. Kuc: None. J. Maguire: None. A. Davenport: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; British Heart Foundation, Wellcome Trust, Medical Research Council.

Funding: No

Funding Component: P184

Co-localization and Natriuretic Interdependence of Angiotensin AT2R and MasR in Obese Rat Kidney

Sanket N Patel, Quaisar Ali, Univ of Houston Coll of Pharmacy, Houston, TX; Ulrike Muscha Steckelings, Univ of Southern Denmark, Odense, Denmark; Tahir Hussain, Univ of Houston Coll of Pharmacy, Houston, TX
The actions of angiotensin II type 2 receptor (AT₂R) and receptor mas (MasR) are complex but show similar pro-natriuretic function; particularly AT₂R expression and natriuretic function are enhanced in obese/diabetic rat kidney. In light of previous reports, we tested hypothesis that AT₂R and MasR are interdependent to produce natriuresis in obese rats due to potential physical interaction. Infusion of AT₂R agonist C21 (5 µg/kg/min) in obese Zucker rats (OZR) caused diuresis/natriuresis which were attenuated by simultaneous infusion of the AT₂R antagonist PD123319 (50 µg/kg/min) or the MasR antagonist A-779 (50 µg/kg/min). Similarly, infusion of MasR agonist Ang-(1-7) (110 fmol/kg/min) in OZR caused diuresis/natriuresis, which were attenuated by simultaneous infusion of A-779 or PD123319. Dual labeling of AT₂R and MasR in OZR kidney slices revealed four-fold co-localization of AT₂R and MasR (9.83 vs. 2.50 dual labeled cells/1600 μm²) compared with lean rats in which AT₂R is not natriuretic. Moreover, the AT₂R co-immunoprecipitates with MasR in cortical homogenate of OZR. Immunoblotting of AT₂R and MasR with zero length oxidative (sulfhydryl groups) cross-linker cupric-phenanthroline in OZR cortical homogenate revealed a shift of AT₂R (~62 kDa) and MasR (~54 kDa) bands upward with overlapping migration for their complexes (~160 kDa and 245 kDa) which were sensitive to the reducing β-mercaptoethanol. Similar observations were made in HK-2 cells, where glucose (25 mM) treatment enhanced the crosslinking. Collectively, the study reveals AT₂R and MasR are co-localized and functionally interdependent in producing natriuretic response. Hyperglycemic oxidative stress affecting sulfhydryl groups present a potential mechanism of such physical interaction between these receptors. (Support: R01DK061578)


Funding: No

Funding Component: P185

Role of Ucp2 in the Oxidative/nitrosative Stress-mediated Hypertension Associated with Dj-1 Depletion

Carmen De Miguel, William C Hamrick, Univ of Alabama at Birmingham, Birmingham, AL; Laureano Asico, Pedro Jose, Santiago Cuevas, The George Washington Univ, Washington DC, DC

DJ-1⁻ mice, relative to wild-type (WT) littermates, have increased blood pressure (BP) (DJ-1⁻: 130±4 vs WT: 100±3 mmHg, n=6/8), and renal expressions of nitro-tyrosine (+76±31% of WT mice, n=5) and malondialdehyde (+63±23% of WT mice, n=4). Tempol, a superoxide dismutase mimetic, decreased the BP of DJ-1⁻ mice (DJ-1⁻: before tempol: 119±3; after tempol: 100±1 mmHg vs WT, n=4) and renal malondialdehyde production (DJ-1⁻: before tempol: +40±5%; after tempol: -24±5% vs WT, n=4) but increased serum nitrate/nitrite levels (+72±30%, n=4), indicating the presence of both oxidative and nitrosative stress. Lack of DJ-1 makes some cells vulnerable to endoplasmic reticulum (ER) stress. However, renal mRNA expression of ER stress markers, GRP94, ATF-4, ATF-6, sXBP-1, CHOP, caspase-12, and caspase-3 was not different between DJ-1⁻ and WT (n=7) mice. Markers of inflammation, IL-6, TNF α, MCP-1, NFkB, and T-cell and macrophage infiltration, were also not increased in the kidney of DJ-1⁻ mice. By contrast, renal mitochondrial (mt) HSP60, but not mtHSP40, was increased in DJ-1⁻ mice (2.9±1.0 fold, n=4) but there were no changes in the renal mRNA expressions of Nix/BNIP3L, BNIP3, PINK, FIS1, MFN1, MFN2, PPRC1, NRF-1, and PGC1, indicating that mt oxidative stress did not affect...
mt function. The renal expression of UCP2, which is involved in the control of mt-reactive oxygen species production, was elevated in DJ-1\(^{-/-}\) mice (4.1±1.1 fold of WT, n=4). Silencing UCP2 in mouse renal proximal tubule cells (-0.46.5±0.01 fold) increased the expression of ER stress and apoptosis markers CHOP (2±0.4 fold), ATF4 (2.6±0.6 fold), caspase-3 (2.3±0.4 fold), and caspase-12 (1.7±0.2 fold)(n=3). There were no differences in renal renin expression, sodium excretion, and serum creatinine between DJ-1\(^{-/-}\) and WT mice (n=5). There were no abnormalities in renal morphology, including fibrosis, in the kidneys of DJ-1\(^{-/-}\) mice. However, urinary KIM-1 was increased in DJ-1\(^{-/-}\) mice (148±22% of WT mice, n=4) and decreased by tempol (-58±3%, n=4); renal UCP2 expression was also partially normalized by tempol (1.8±0.2 fold of WT, n=4). UCP2 may protect from the development of renal ER stress and damage in the mt oxidative/nitrosative stress associated with DJ-1 depletion.

C. De Miguel: None. W.C. Hamrick: None. L. Asico: None. P.A. Jose: None. S. Cuevas: None. Funding: No

Funding Component: P186

Proximal Tubular-Specific Overexpression of the Cyp4a12-20-Hete Synthase Promotes Hypertension

Ankit Gilani, Varunkumar Pandey, Rebecca Hutcheson, Katherine Gotlinger, Danielle Astarita, Michal Schwartzman, New York Medical Coll, Valhalla, NY

20-Hydroxyeicosatetraenoic acid (20-HETE) is a cytochrome P450 (CYP)-derived arachidonic acid metabolite linked to regulation of blood pressure via actions on the renal microvasculature and tubules. In renal microvessels, 20-HETE promotes vasoconstriction, vascular remodeling, and vascular injury; all of which contribute to hypertension, whereas in tubular structures 20-HETE has been linked to inhibition of sodium transport and lowering of blood pressure. Induction or global overexpression of Cyp4a12-20-HETE synthase, the primary 20-HETE producing enzyme in mice, results in hypertension. Since these mice display global overproduction of 20-HETE, it is difficult to dissect the exact contribution of vascular versus tubular 20-HETE to the hypertension. To begin addressing this issue, the current study was undertaken to define the blood pressure phenotype of novel transgenic mice with proximal tubule-specific overexpression of the Cyp4a12-20-HETE synthase (PEPCK-cyp4a12 mice). These mice generated by crossing the Cyp4a12\(^{fl/fl}\) with PEPCK (phosphoenolpyruvate carboxykinase promoter)-Cre mice, showed increased Cyp4a12 mRNA expression (2.6±0.9 fold increase; p<0.05) and 20-HETE production in the renal cortex (138±32 pg/mg vs. 80±6 pg/mg). Most importantly, the systolic blood pressure was markedly elevated in the PEPCK-Cyp4a12 mice as compared to WT littermates (142±2 vs. 111±3 mmHg; p<0.05). Interestingly, the PEPCK-Cyp4a12 mice exhibited significantly higher levels of urinary 20-HETE as compared to WT littermates (205±50 vs 83±5 pg/ml; p<0.05) whereas plasma 20-HETE levels remained unchanged (231±32 vs 190±25 pg/ml). Moreover, in PEPCK-Cyp4a12 (n=4) urinary volume was lower (2.15±0.05 vs 3.17±0.20 ml/day, p=0.003) and UNaV appeared lower (181±7 vs 194±25 µmol/day, p=0.35) as compared to WT littermates. These results are in line with the possibility that 20-HETE overproduction at the level of the proximal tubule promotes rather than opposes hypertension. The exact mechanisms need to be further examined.

Partial Renal Infarction Induces Hypertension

Jie Zhang, Jin Wei, Gensheng Zhang, Shaohui Wang, Lei Wang, Univ of South Florida, Tampa, FL; Jacantha Buggs, Tampa General Hosp, Tampa, FL; Ruisheng Liu, Univ of South Florida, Tampa, FL

Renal infarction is an under-diagnosed and under-reported phenomenon. The U.S. incidence of renal infarction is estimated at 1.4%. Systemically thromboembolic originate from thrombus in the heart or aorta while renal infarction in situ typically involves the main renal artery or its branches. Acute or aggravated hypertension is commonly observed in previously normotensive or hypertensive patients with renal infarction. However, these pathophysiological mechanisms have not been elucidated. The goal of this study was to develop a hypertensive mouse model of renal infarction. Partial renal infarction was performed in C57BL/6 mice by ligating either the upper (LU) or lower (LL) branch of the renal artery in the left kidney while the right kidney remained intact. The mean arterial pressure (MAP) was continuously measured with a telemetry system in conscious mice fed 4 weeks of normal salt diet (NS) (0.4% NaCl) followed by 4 weeks of high salt diet (HS) (4% NaCl). Plasma renin concentration (PRC), renin mRNA in the kidney and TNF-α were measured. Body weight, salt and fluid intakes were similar in mice with LU and LL ligation compared with sham operated mice. The weight of the left kidney decreased by 16.3% in LU (118.1±8.9 mg) and 14.2% in LL (121.5±7.8 mg) compared with sham operated mice (141.0±9.5 mg) (n=6; p<0.05 vs sham). The right kidney weight increased by 41.5% in LU (201.3±15.6 mg) and 38.2% in LL (196.6±8.1 mg) compared with sham mice (142.2±8.8 mg) (n=6; p<0.01 vs sham). MAP in mice fed NS elevated by 25% in LU (119.4±12.9 mmHg) and 19.1% in LL (113.7±10.6 mmHg), compared with sham (95.4±4.7 mmHg) (n=4; p<0.05 vs sham). HS further increased the MAP to 124.2±17.4 mmHg in LU and 118.6±14.8 mmHg in LL mice. PRC decreased by 50.0% in LU (30.7±8.63 ng/ml) and 62.7% in LL (22.9±10.8 ng/ml), compared with sham operated mice (61.4±12.6 ng/ml) (n=6; p<0.05 vs sham). Expression of local renin mRNA in the left kidney was upregulated by 113.4% in LU and 64.1% in LL mice, compared with the sham. Inflammatory cytokines TNF-α was increased by 174.2% in LU and 106.3% in LL mice. In conclusion, we developed a mouse model of partial renal infarction with hypertension in C57BL/6 mice. The mechanism of hypertension may be due to the upregulation of local renin angiotensin system and inflammation.


Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

(Pro)renin Receptor (PRR) Mediates High Fat Diet-induced Hypertension via Upregulation of Epithelial Sodium Channel & Aquaporin-2

Syed S Quadri, Silas A Culver, Helmy M Siragy, Univ of Virginia, Charlottesville, VA

We hypothesized that the renal prorenin receptor (PRR) mediates high fat diet (HFD)-induced hypertension via enhancing expression of epithelial sodium channel (α-ENaC) and aquaporin-2 (AQP-2). C57BL/6 mice underwent right nephrectomy (n=12) and allocated to
receive regular diet (RD, 12 kcal% fat, n=6) or a high-fat diet (HFD, 45 kcal% fat, n=6) for 10 weeks. HFD group received left renal subcapsular interstitial administration of PRR shRNA (n=3) or vehicle (n=3). BW, food and water intake, BP, UV and UNaV, renal interstitial fluid (RIF) angiotensin II (Ang II), and renal expressions of PRR, α-ENaC, AQP-2 were monitored. At baseline, there were no significant differences in BW, food and water intake, BP, UV and UNaV between different animal groups. At the end of the study, HFD mice had significant increase in food intake, systolic blood pressure (HFD 154.6 ± 2.181 mmHg, vs. RD 112.21 ± 4.684 mmHg, P<0.0.05), BW (HFD 43.2 ± 1.125 gm, vs. RD 31.9 ± 0.89654 gm, P<0.0.05), and significant reduction in UV (HFD 0.16 ± 0.042 ml, vs. RD 0.41 ± 0.061 ml, P<0.0.05) and UNaV (HFD 26.02 ± 3.652 µmol/day vs. RD 46.32 ± 5.236 µmol/day, P<0.0.05). Compared to RD, there were significant increases in mRNA and protein expressions of PRR (64% and 40%, P<0.01), α-ENaC (85% and 75%, P<0.05), AQP-2 (105% and 80%, P<0.05) in HFD alone mice respectively. Compared to HFD alone, HFD+ PRR shRNA treatment caused significant reductions in BP (108 ± 13.88 mmHg vs HFD 154.6 ± 2.181 mmHg), mRNA and protein expressions of PRR (33% and 50%, P<0.01), α-ENaC (49% and 56%, P<0.05), AQP-2 (30% and 29%, P<0.05) respectively. There were no changes in RIF Ang II between different animal groups. We conclude that PRR mediates HFD-induced hypertension via enhancing renal α-ENaC and AQP-2 expression independent of Angiotensin II.

S.S. Quadri: None. S.A. Culver: None. H.M. Siragy: None.

Funding: No

Funding Component: P189
of EET in the regulation of NCC expression is further suggested by the finding that high fat-diet induced increase in NCC expression is inhibited by application of EET. We conclude that CYP2c44-derived EET plays an important role in inhibiting NCC and ENaC in the DCT.


Funding: No

Funding Component: P190

Alamandine Improves Cardiac Post-Ischemic Function in Isolated Hearts of TGR(mREN2)27

Jônatãs F. Almeida, Robson A. Santos, Federal Univ of Minas Gerais, Belo Horizonte, Brazil

The renin-angiotensin system (RAS) is an important regulator of cardiovascular function. Over the past years, new peptides have been described in this system. Among these new peptides, Alamandine was recently described by Santos and colleagues as an important participant in the control of cardiovascular function through its interaction with MrgD receptors. Our previous studies showed that Alamandine improved post-ischemic cardiac function in normotensive rats. Here we assessed the effects of Alamandine in a more challenging condition using hypertensive rats. TGR(mREN2)27 rats weighing between 250-300g were euthanized and their hearts were placed on Langendorff apparatus to evaluate the cardiac parameters. Hearts were submitted to 30min of stabilization, 30min of partial ischemia by occlusion of the left descending coronary artery and 30min of reperfusion. Alamandine (ALA, 22pM) was added to the perfusion setting from the beginning of the experiment until the end. 2,3,5-triphenyltetrazolium chloride were used to evaluate the extension of infarcted area. In hypertensive (mREN) animals there was an impairment in the baseline (35.57 ± 5.839 mmHg) of left ventricular systolic pressure (LVSP), and this impairment was attenuated by ALA (54.91 ± 6.304mmHg). Maximum and minimum dp/dt in mREN presented a significant reduction in the ischemic period (926.5 ± 172.3 mmHg/s and 682.7 ± 106.1 mmHg/s, respectively) compared to baseline (1761 ± 219.7 mmHg/s and 1242 ± 150.9 mmHg/s, respectively). Ischemia/reperfusion induced an arrhythmia severity index (ASI) in mREN hearts higher than in hearts treated with ALA (4,9 ± 1,26) vs (1,10 ± 0,58). ALA also reduced infarcted area compared to mREN (19,64 ± 2,61%) vs (33,85 ± 4,55%). In conclusion, our data shown that Alamandine exert cardioprotective effects in post-ischemic function in hypertensive rat isolated hearts by preventing LVSP, dp/dt max and min decrease. Furthermore, it reduced the infarcted area and I/R arrhythmias.

J.F.Q. Almeida: None. R.A.S. Santos: None.

Funding: No

Funding Component: P191

The Origin and Fate of Renin Progenitors During Hematopoiesis

Brian C Belyea, Fang Xu, Maria Luisa S Sequeira-Lopez, R. Ariel Gomez, Univ of Virginia, Charlottesville, VA

Our lab previously discovered the presence of novel renin-expressing progenitors within the hematopoietic system. These progenitors have cell surface markers, gene expression, and growth characteristics of B lymphocytes. Further, these cells represent a subset of total B lymphocytes, are numerous at birth, and diminish with age, suggesting renin expression may be prominent during embryonic
hematopoiesis. However, it is unknown when renin progenitors first appear and what function they serve during hematopoietic development. In this study, we sought to further define the temporal appearance, identity, and evolution of renin progenitors throughout hematopoietic ontogeny. We used in vivo lineage-tracing techniques, flow cytometry, immunofluorescence, and polymerase chain reaction (PCR) analysis to investigate the origin and fate of renin hematopoietic progenitors. We found that renin expressing hematopoietic progenitors first appear within the yolk sac during mid gestation (E11.5 by PCR and E12.5 by flow cytometry) and peak in number at E13.5 (14.9 ± 4.8% of nucleated single cells by flow cytometry). Subsequently, renin lineage cells leave the yolk sac and colonize the fetal liver and spleen at E15.5. In the fetal liver and fetal spleen, renin lineage cells express B cell surface markers including CD19 and CD43, however they have dim B220 expression, consistent with a B-1 progenitor immunophenotype. Renin lineage cells within the bone marrow, spleen, and peripheral blood peak in number shortly after birth and then decrease with post-natal age and have a phenotype consistent with B-2 B lymphocytes (B220+CD19+CD23+CD11b-).

Conversely, renin progenitors in the peritoneal cavity persist throughout adult life as B-1 B cells (B220dimCD19+CD23+CD11b+). These studies suggest that renin progenitors originate within the yolk sac during the initial wave of primitive B lymphopoiesis and then expand to the fetal liver and spleen prior to the development of definitive hematopoiesis. Renin-lineage cells persist during adult life as B-1 B cells in the peritoneal cavity and, to a lesser extent, as B-2 B cells in the bone marrow, spleen, and peripheral blood. The function of these renin progenitors is currently being investigated.


Funding: No

Funding Component: P192

**AT1R Blockade Increases the Depressor Effect of Alamandine in Normotensive SD Rats**

**Giovanni Canta**, UFMG, Belo Horizonte, Brazil; Roberto Lautner, UFJF, governador valadares, Brazil; Robson Santos, UFMG, Belo Horizonte, Brazil

The renin–angiotensin system (RAS) plays a critical role in blood pressure control and electrolyte homeostasis. It has been established that Ang II is not the only active peptide of the RAS. Recently, new active fragments formed through metabolism of the classical axis peptides have been identified and their biological effects characterized. One of these peptides, is Alamandine, which was described by our group. Here we investigate the effect of Alamandine on blood pressure in non-anesthetized SD rats. To study the in vivo effects of this peptide, we used 12 weeks old SD rats (300-400 g). Administration of drugs and the blood pressure measurement were performed with a cannula inserted through the femoral vein and artery, respectively. We administered Alamandine with bolus injections (0.02, 0.2, 1, 5, 20 and 80 ng). A biphasic effect of alamandine was observed. The greatest depressor effect of Alamandine was observed at a lower dose, 1 ng. (Δ MAP: -10.33±0.85 mmHg $p<0.05$). On the other hand, the highest dose, 80 ng, did not change significantly the blood pressure. Interestingly, when Losartan (5mg/kg) was administered 30min before the injection of alamandine, the depressor effect of this peptide was increased, mainly at 0.2 ng (Δ MAP: -6±1.05 mmHg Alamandine vs. Δ MAP: -20.33 ± 2.34 mmHg Alamandine after Losartan $p<0.05$). In losartan treated rats only depressor response were observed with all doses. In conclusion, our data show that Alamandine has...
a biphasic effects on blood pressure and seems to interact with the AT1 receptor in a dose-dependent manner.

G. Canta: None. R. Lautner: None. R. Santos: None.

Funding: No

Funding Component: P193

Platelet-derived Growth Factor B: a Novel Determinant of Juxtaglomerular Cell Phenotypic Plasticity?

Alexandre Goes Martini, Univ of Brasilia, Brasilia, Brazil; Edith C Friesema, Alexander H Danser, Erasmus MC, Rotterdam, Netherlands; Tim L Reudelhuber, Inst de Recherches Cliniques de Montréal, Montreal, QC, Canada

Renin, a key component in the regulation of blood pressure in mammals, is produced by the rare and highly specialized juxtaglomerular (JG) cells of the kidney. JG cells may be derived from vascular smooth muscle cells (VSMC) and they can reversibly differentiate in response to changes in salt, blood pressure and treatment with anti-hypertensive medications. The biochemical mechanism responsible for this phenotypic plasticity is currently unknown. Ligands involved in VSMC differentiation include platelet-derived growth factor B (PDGF-B), secreted acidic protein rich in cysteine (SPARC), type-C natriuretic peptide (NPPC), follistatin related protein (FSTL1) and lactose-binding lectin 1 (LGALS1). To test for the role of these factors in JG cell plasticity we used (pro)renin-producing As4.1 cells derived from a mouse JG cell targeted tumor. As4.1 cells were incubated for 48 hours with conditioned medium derived from human embryonic kidney (HEK) 293 cells transfected with the mouse cDNA encoding these ligands, after which both medium and cell lysate were collected. Renin and prorenin were measured using the angiotensin I generation assay. Under control conditions, the medium contained predominantly (>95%) prorenin. In contrast, the cell lysate contained renin only, at levels corresponding to <1% of the total amount of renin+prorenin in the medium (i.e., 161±61 μg angiotensin I/ml.hr, mean±SEM). Among the tested ligands, only PDGF-B affected the medium prorenin and cellular renin levels, decreasing both in parallel by 68±5% and 53±10%, respectively. In addition, PDGF-B-exposed cells changed their morphology to display a more elongated, densely packed and aligned shape with no apparent alteration in their viability. In conclusion, our data suggest that PDGF-B might be one of the factors involved in JG cell phenotypic plasticity.


Funding: No

Funding Component: P194

A New Role of Sox6 in Blood Pressure Through Renin Regulation

Jose A Gomez, Vanderbilt Univ Medical Ctr, Nashville, TN; Conrad P. Hodgkinson, Alan Payne, Duke Univ, Durham, NC; David G. Harrison, Vanderbilt Univ Medical Ctr, Nashville, TN; Victor J. Dzau, Duke Univ, Durham, NC

Hypertension afflicts 33% of the U.S. adult population. Despite current treatments, approximately 50% of people are unresponsive to treatment. There is a critical need to develop new therapies to treat this disease and its complications. The Renin Angiotensin Aldosterone System plays a key role in regulating blood pressure in humans. Renin controls the rate-limiting step in the conversion of angiotensinogen to angiotensin I. In adults, renin is produced and stored by Juxtaglomerular (JG) cells in the kidney.
However, the transcriptional mechanisms that govern formation of renin expressing cells under normal or pathophysiological conditions remain poorly understood. During blood pressure changes the number of adult renal cells expressing renin increase through a process known as JG cell expansion. We found that this process involves differentiation of mesenchymal stromal-like cells (MSC) to renin expressing cells among others. We aim to determine new regulators of renin expression and blood pressure control. Renin expression in vitro was induced by treatment of MSCs with 3-isobutyl-1-methylxanthine (IBMX) and Forskolin. MSCs were transduced with lentivirus carrying vectors Sox6 shRNA or control shRNA. A new transgenic mouse, in which Sox6 is deleted specifically in renin expressing cells (Ren1dCre/Sox6fl/fl), was used to study the impact of Sox6 in renin expression in vivo. Gene array comparing renal MSC and JG cells identified potential genes that control MSC differentiation, including Sox6. In vitro silencing of Sox6 by shRNA decreased differentiation of renal MSCs into renin producing cells (3.5 fold, n=4, P= 0.01). Preliminary IHC data showed that the transcription factor Sox6 is expressed by renin producing cells in the adult kidney during JG cell expansion. Plasma renin concentration (PRC) increased during JG cell expansion (induced with low sodium diet and furosemide) in wild type mice (27-fold), whereas in mice lacking Sox6 in JG cells PRC was as low as non-treated mice. We conclude that Sox6 has a novel role in modulating renin expression and thereby can contribute to renal physiology, opening new possibilities of addressing questions regarding physiological regulation of renin and hypertension.

J.A. Gomez: None. C.P. Hodgkinson: None. A. Payne: None. D.G. Harrison: None. V.J. Dzau: None.

Funding: No

Funding Component: P195

Prorenin Receptor Activation Increases Profibrotic Markers and Fibroblast Like Phenotype Through MAPK-dependent ROS Formation in Mouse Renal Collecting Duct Cells

Alexis A Gonzalez, Cristian Reyes-Martinez, Leonardo Zamora, Pontifical Catholic Univ of Valparaiso, Valparaiso, Chile; Minolfa C Prieto, Tulane Univ, New Orleans, LA

Binding of prorenin or renin to (pro)renin receptor (PRR) activates mitogen activated protein kinases (MAPK)/extracellular regulated kinases 1/2 (ERK 1/2), which have been proposed as mediators in tissue damage. Prorenin, renin, and PRR are overexpressed in the collecting duct (CD) in diabetes and hypertension. Activation of the PRR upregulates profibrotic markers through reactive oxygen species (ROS) formation; however, the exact mechanisms have not been established. We hypothesized that the activation of PRR increases the expression of profibrotic markers through MAPK-dependent ROS formation in CD cells. To address this hypothesis, a mouse renal CD cell line (M-1) was treated with recombinant prorenin plus ROS or MAPK inhibitor to evaluate their effect on the expression of profibrotic markers. Recombinant prorenin increased ROS formation and expression of alpha smooth muscle actin (α-SMA), connecting tissue growth factor (CTGF) and plasminogen activator inhibitor-1 (PAI-1). Induction of profibrotic factors was blunted by antioxidant p-coumaric acid, and partially prevented by lyophilic antioxidant trolox C or ascorbic acid. Inhibition of MAPK pathway (PD98059) prevented the prorenin-dependent ROS formation and augmentation of profibrotic factors, as well as fibroblast-like phenotype. Knockdown of PRR did not show effects on cell viability in M-1 cells and partially reduced the augmentation of fibronectin, collagen I and fibroblast-like
phenotype induced by prorenin treatment. These results suggest that the activation of CD-derived PRR plays a role in the development of tubular damage.

A.A. Gonzalez: None. C. Reyes-Martinez: None. L. Zamora: None. M.C. Prieto: None.

Funding: No

Funding Component: P196

Effects of the Physiological Increase in Angiotensin-converting Enzyme in Acute Myocardial Infarction

Oscar A Moraes, Heart Intitute, Univ of São Paulo, Medical Sch, São Paulo, Brazil; Leonardo Jensen, Heart Inst, Univ of São Paulo, Medical Sch, São Paulo, Brazil; Leandro E Souza, Maikon Barbosa, Kátia B Scapini, Heart Intitute, Univ of São Paulo, Medical Sch, São Paulo, Brazil; Cristiano T Mostarda, Federal Univ of Maranhão, São Luis, Brazil; Maria C Irigoyen, Heart Inst, Univ of São Paulo, Medical Sch, São Paulo, Brazil

The angiotensin converting enzyme (ACE) act in the production of ANG II and degradation of bradykinin. It is believed that higher levels of ACE could modulate susceptibility to coronary occlusion. To investigate this possibility we used adult males mice genetically modified to express different concentrations of ACE similar to that found in humans. Animals (1, 2 or three copies of ACE gene; n = 6 each group) were subjected to acute myocardial infarction (AMI) and evaluated 7 days after. Systolic, diastolic, and mean blood pressure (BP), heart rate, heart rate variability (HRV) were evaluated as well as cardiac function by echocardiography.

Regarding hemodynamic data, AMI induced no difference in systolic, diastolic and mean BP of different gene copies mice (ACE1: 74.02 ± 3.0; ACE2: 108.65 ± 2.8; ACE3: 102.07 ± 4.1mmHg). Regarding heart rate it was observed a reduction in 3 copies group (ACE1: 654.51 ± 26.34; ACE2: 668.22 ± 10; ACE3: 534.11 ± 33.4 BPM). The heart rate variability evaluated by SDNN (ACE1: 5.16 ± 0.93; ACE2: 5.84 ± 1.32; ACE3: 7.69 ± 1.4 ms), RMSSD (ACE1: 5.91 ± 0.69; ACE2: 5.7 ± 1:14; ACE3: 6.78 ± 1.2ms), and the total variance (ACE1: 30 ± 12.4; ACE2: 37 ± 13.8; ACE3: 69.9 ± 25.6 ms) was higher in the ACE 3 infarcted group. Regarding the spectral analysis of HRV a higher sympathetic modulation (ACE1: 5.14 ± 3; ACE2: 7.2 ± 2.8; ACE3: 12.9 ± 4.1ms²) and a lower parasympathetic modulation (ACE1: 7 ± 2; ACE2: 8.6 ± 3.2; ACE3: 5.5 ± 2.8 ms²) were observed in ACE 3 infarcted mice.

The variability of the BP (ACE1: 18.7 ± 6.2; ACE2: 14.85 ± 1.6; ACE3: 13.76 ± 4.2 mmHg²) and the sympathetic modulation component of the vessels were reduced in the ACE 3 AMI group (ACE1: 60.5 ± 6.2; ACE2: 54.6 ± 1.7; ACE3: 53 ± 3.3 mmHg²). Echocardiographic data showed diastolic dysfunction expressed by E / A ratio (ACE1: 3.40 ± 0.67; ACE2: 3:02 ± 1:36; ACE3: 2.0 ± 0.75) and increase in FAC - Fractional Area Change (ACE1: 27.9 ± 4.5; ACE2: 26.4 ± 5.7; ACE3: 33.5 ± 1.3) in the ACE 3 group after AMI. Our data suggest that increasing the number of ACE gene copies may promote autonomic imbalance evidenced by increased sympathetic modulation and the reduction of parasympathetic modulation of the cardiovascular system as well as the impairment of the diastolic function.


Funding: No

Funding Component: P197

Differences in Renin-angiotensin System Components Expression in Tumoral and Normal Cell Lines
Several reports have shown the actions of ACE/AngII/AT1 axis in the development of malignancy and also predict that RAS inhibitors could potentiate cancer therapies. On the other hand, the alternative axis of renin angiotensin system, ACE2/Ang-(1-7)/Mas, demonstrated an anti-tumoral property. This anti-tumoral effect is not clear for the recently described components of RAS, Alamandine/MrgD. The aim of this work is to characterize the expression and the enzyme activities of the RAS components in different tumoral and normal cell lines. There was a significant increase in the expression of the components (ACE and AT2) related to the formation and actions of Ang II. ACE presented 1.8 A.U. and 1.4 A.U. in A549 and MIAPACA tumoral cell lines, respectively, in comparison with 0.6 A.U. of normal cell lines (VERO) (p< 0.05). On the other hand, there was reduction in ACE2 enzyme related to the formation of Angiotensin-(1-7) and Alamandine (0.25 A.U. in MIAPACA VS 1.7 A.U. in VERO, p<0.05), which are modulatory peptides of the actions of Angiotensin II. Furthermore, there is also a significant increase in both Mas (0.18 A.U. in A549 VS 0.12 A.U. in VERO, p<0.05) and MrgD receptors (85 ±6 A.U. in A549 and 73 ±5 A.U. in MIAPACA VS 11 ±3 A.U. in VERO, p<0.001) expression in tumoral cells. Additionally, to complete the data obtained by Western Blotting, the enzymatic activity of ACE and ACE2 was evaluated in tumor cells and in Vero cells used as control. ACE enzyme activity is increased in tumor cells, while the ACE2 is reduced (950 A.U. in VERO VS 500 A.U. MIAPACA and 450 A.U. A549 p<0.01). These data suggest that RAS proliferative axis is activated in tumor cells and anti -proliferative axis is decreased compared to control cells.
intake (15.02±1.3 kcal/day and 15.4±0.8 kcal/day). Plasma glucose, area under curve (AUC) of GTT, and HOMA-IR were significantly increased in HF mice (121±6mg/dL; 32,865±6,222; 5±1) compared to CT mice (220±27mg/dL; 55,549±4,611; 54±9). In HF mice, SBP was augmented by wk 10 compared to CT mice (126±5 vs. 113±1 mmHg; P<0.05). Interestingly, no differences in albuminuria were found between HF and CT mice either at wk 12 (50±13 vs. 40±6 ug/day) or at wk 24 (57±7 vs. 43±3 ug/day). However, uAGT excretion was increased by wk 24 in HF mice but not in CT mice (wk 0: 4±1.5 vs. 4±1.5 ng/day; wk 12: 4.6±2.3 vs. 2.3±0.9 ng/day; wk 24: 11.8±2.9 vs. 3.9±0.3 ng/day; P<0.05). During HF diet induced-type 2 DM, the elevation of uAGT reflects the increase of intrarenal RAS which may contribute to the development of renal injury.


Funding: No
Funding Component: P199

Mas Receptor Deficiency Regulates Obesity-Hypertension and Cardiac Function in Female and Male Mice

Robin C Shoemaker, Yu Wang, Sean Thatcher, Lisa Cassis, Univ of Kentucky, Lexington, KY

Angiotensin-1-7 (Ang-(1-7)) counteracts angiotensin II through effects at Mas receptors (MasR). We demonstrated that sexual dimorphism of obesity-hypertension was associated with dysregulated production of Ang-(1-7). However, the role of MasR in sexual dimorphism of obesity-hypertension has not been examined. MasR deficient mice have also been reported to exhibit deficits in cardiac function. In this study, we hypothesized that deficiency of the MasR would differentially regulate obesity-hypertension in male versus (vs) female mice. In addition, we quantified effects of MasR deficiency on cardiac function in obese male mice. Male and female MasR+/+ and +/- mice were fed a low fat (LF, 10% kcal) or high fat (HF, 60% kcal) diet for 16 weeks, and blood pressure was quantified by radiotelemetry. As demonstrated previously, male MasR+/+ mice (24 hr diastolic blood pressure, DBP: LF, 90 ± 3; HF, 96 ± 2 mmHg; P<0.05), but not females (LF, 85 ± 1; HF, 85 ± 2 mmHg), developed hypertension in response to HF feeding. MasR deficiency converted female HF-fed mice to an obesity-hypertension phenotype (DBP: 92 ± 1 mmHg; P<0.05). Surprisingly, male HF-fed MasR+/+ mice exhibited reduced DBP compared to HF-fed MasR+/+ males (90 ± 1 vs 96 ± 2 mmHg; P<0.05). To define mechanisms for reductions in DBP of HF-fed male MasR+/+ mice, we performed cardiac magnetic resonance (CMR) imaging in both genotypes at 1 month of HF feeding. MasR+/+ mice had significantly reduced ejection fraction (EF) compared to MasR+/+ mice at baseline (51.4 ± 2.5 vs 59.3 ± 2.1%; P<0.05) and after one month of HF-feeding (49.8 ± 2.4 vs 52.6 ± 1.9%; P<0.05). Further, CMR imaging demonstrated a thickening of the ventricle wall in MasR+/+ mice with 1 month of HF-feeding. MasR+/+, but not MasR+/+ mice, exhibited diet-induced reductions in EF (by 16%; P<0.05) at 1 month of HF feeding, which were reversed by infusion of Ang-(1-7). These results demonstrate that MasR contributes to sexual dimorphism of obesity-hypertension. Ang-(1-7) protects females from obesity-hypertension through the MasR. In contrast, reductions in DBP in obese male mice with MasR deficiency may arise from deficits in cardiac function. These results suggest that MasR agonists may be effective therapies for obesity-associated cardiovascular conditions.

Funding: Yes
Funding Component: Great Rivers Affiliate
(Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)
P200

Role of Macula Densa Neuronal Nitric Oxide Synthase in Control of Renin Release and Blood Pressure Recovery Following Hemorrhagic Shock

Lei Wang, Shaohui Wang, Univ of South Florida, Tampa, FL; Jacentha Buggs, Tampa General Hosp, Tampa, FL; Jie Zhang, Jin Wei, Gensheng Zhang, Ruisheng Liu, Univ of South Florida, Tampa, FL

Renin is a rate limiting factor for generation of angiotensin II, which is essential for blood pressure regulation. The role of macula densa nitric oxide (NO) in renin release is not conclusive. The goal of this study was to elucidate the role of macula densa neuronal NO synthase (NOS1) in control of renin release in response to sodium challenges and hemorrhagic shock, as well as in blood pressure recovery after hemorrhagic shock.

C57BL/6L mice and macula densa specific NOS1 knockout (MD-NOS1KO) mice were given 10 days of low (0.1% NaCl), normal (0.4% NaCl) and high (1.4% NaCl) sodium diet. Hemorrhagic shock was induced by withdrawing 0.4 ml whole blood from the right retro-orbital sinus. Mean arterial pressure (MAP) in conscious mice was monitored by radio-telemetry system. Plasma renin concentration (PRC) was determined by radioimmunoassay.

Low sodium diet stimulated PRC by 29% (from 685 ± 32 to 883 ± 112 ng/ml/hr) in WT mice and by 16% (from 652 ± 24 to 756 ± 124 ng/ml/hr) in the MD-NOS1KO mice (n=5/group, p<0.01 vs WT). PRC was not significantly different between the WT and MD-NOS1KO mice fed a normal or high salt diet. As shown in Fig1A, following removal of 0.4ml of blood, MAP dropped to about 40mmHg in the WT mice and 35mmHg in the MD-NOS1KO mice. MAP recovered faster in WT mice than the MD-NOS1KO mice. In Fig1B, PRC increased over 200% of the basal value in WT mice, but only increased about 26% in the MD-NOS1KO mice (n=4/group, p<0.01 vs WT).

We conclude that NOS1 in the macula densa facilitates renin release. Lack of macula densa NO generation impairs blood pressure recovery, which may be mediated by limiting renin release during hemorrhagic shock.


Enhanced Upregulation of (Pro)renin Receptor Expression by High Salt Intake in the Kidney of Dahl Salt-sensitive Rats

Seiko Yamakoshi, Osamu Ito, Rong Rong, Yusuke Ohsaki, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Yoshikazu Muroya, Tohoku Medical Pharmaceutical Univ Sch of Med, Sendai, Japan; Takefumi Mori, Sadayoshi Ito, Kazuhiro Takahashi, Tohoku Univ Graduate Sch of Med, Sendai, Japan; Kazuhiro Totsune, Tohoku Fukushi Univ, Sendai, Japan; Masahiro Kohzuki, Tohoku Univ Graduate Sch of Med, Sendai, Japan
We recently reported that high salt (HS) intake increased the (pro)renin receptor ((P)RR) expression by 3-5 fold in several nephron segments of Sprague-Dawley rats (Peptides 63: 156-162, 2015). The preset study examined the effects of HS intake on the renal (P)RR expression in Dahl-Salt sensitive (DS) rats. Male DS rats were fed a normal salt (NS) diet (0.6%NaCl) and a HS diet (8%NaCl) for 4 weeks. A part of the rats fed the HS diet were treated orally with angiotensin II type 1 receptor (AT1R) antagonist, candesartan (Can, 3mg/kg/day) or mineralocorticoid receptor (MR) antagonist, spironolactone (Spi, 100mg/kg/day). The (P)RR expression in nephron segments was examined by immunoblot and immunohistochemical analyses. HS intake increased the blood pressure, which did not significantly affected by Can or Spi. (P)RR was expressed in the all kidney sections, glomeruli, proximal tubules (PT), medullary thick ascending limbs and inner medullary collecting ducts. HS intake increased the (P)RR expression in the cortex by 22.6 fold (p<0.001) and the PT by 4.9 fold (p<0.01), but did not change it in the other sections or segments. Can inhibited the HS intake-increased (P)RR expression in the cortex by 32% (p<0.05), Spi inhibited it by 89% (p<0.001), but neither drug did not inhibit the HS intake-increased (P)RR expression in the PT. Immunohistochemical analysis also revealed that HS intake increased the (P)RR expression in the PT and distal tubules, and that Can and Spi inhibited the HS intake-increased (P)RR expression in the distal tubules. Additionally, deoxycorticoesterone acetate (DOCA, 50mg/kg/week) administered to rats fed the NS diet for 4 weeks increased the (P)RR expression in the cortex by 80% (p<0.001) and distal tubules, but not in the PT. These results indicate that HS intake-increased (P)RR expression is enhanced in the PT and distal tubules of DS rats. The mechanisms of HS intake-increased (P)RR expression may be AT1R and MR-dependent manner in the distal tubules, but AT1R or MR-independent manner in the PT.


Funding: No

Funding Component: P202

Impaired Central, Renal, and Blood Pressure Responses to Alterations in Fluid and Electrolyte Homeostasis in Aged Sprague-Dawley Rats

Alissa A Frame, Kyle R Rodrigues, Lillian M Whelan, Richard D Wainford, Boston Univ, Boston, MA

Aim Hypertension is strongly correlated with increased age in human subjects. These studies tested the hypothesis that impaired natriuretic responses to acute and chronic challenges to fluid and electrolyte homeostasis contribute to age-dependent hypertension.

Methods Two-month old (young) and 6-month old (aged) male Sprague-Dawley rats underwent an acute IV volume expansion (VE; 5% BW) and MAP, HR, urine output, and PVN neuronal activation (c-Fos expression) were assessed (n=4 per group). In a separate study, naïve 2 and 6-month old male Sprague-Dawley rats were maintained on a normal (0.6% NaCl) or high salt (4% NaCl) diet, and on day 21, MAP and NCC activity (peak natriuresis to IV HCTZ, 2mg/kg) were assessed (n=4 per group).

Results Renal excretion of sodium and water after acute VE was impaired in aged rats (total % sodium load excreted; young 78±6 vs aged 60±7: total % water load excreted; young 96±7 vs aged 66±5, P<0.05). PVN neuronal activation was attenuated in response to acute VE in aged rats in all parvocellular regions excluding the lateral parvocellular subnucleus (PVN neuronal
activation [c-fos positive cells]; medial parvocellular young 59±4 vs aged 42±7, P<0.05). CV parameters did not change in response to acute VE in either group. Chronic HS diet evoked an increase in MAP in aged rats but not young rats (MAP [mmHg]; young NS 124±2 vs young HS 126±3 vs aged HS 138±3, P<0.05). Chronic HS diet caused a decrease in NCC activity in young rats and, in contrast, an increase in aged rats (peak ΔUNaV to HCTZ [μeq/min]; young NS 9.2±0.5 vs young HS 7.1±0.3 vs aged HS 16±1, P<0.05).

**Conclusion** Activation of sympathoinhibitory PVN parvocellular neurons is a well-characterized response to acute VE. Our data suggest there are attenuated acute PVN sympathoinhibitory responses to alterations in fluid and electrolyte balance in aged rats. In aged animals, HS intake increased NCC-mediated sodium reabsorption and promoted the development of sodium-dependent hypertension. We speculate that this is driven by blunted HS-evoked sympathoinhibition, likely due to reduced PVN neuronal activation.

**Funding:** No

**Funding Component:** P203

**Renal Localization of Salt Inducible Kinase-1 and Its Regulation in Doca/salt Hypertensive Rats**

**Sabrina R Gonzalez,** Fernanda M Ferrão, Federal Univ of Rio de Janeiro, Rio de Janeiro, Brazil; Minolfa C Prieto, Tulane Univ, New Orleans, LA; Lucienne S Lara, Federal Univ of Rio de Janeiro, Rio de Janeiro, Brazil

In immortalized and primary cultured renal cells, SIK-1 is the intracellular Na⁺ sensor enabling cells to enhance (Na⁺+K⁺)ATPase (NKA) activity without promoting cell swelling. In the present study, we aimed to: (1) Localize SIK-1 along the nephron; (2) To determine the effects of high salt (HS) diet and hypertension (HTN) on SIK-1 expression and activity; and (3) To examine the SIK-related impact on NKA. Adult male Wistar rats (N=60; N=15/group) underwent to unilateral nephrectomy and divided in four groups: 1) Control (CTRL); 2) CTRL/Salt (4% NaCl); 3) Deoxycorticosterone acetate (DOCA; sc 8 mg/kg; twice a week); and 4) DOCA/Salt. After 4 weeks, systolic blood pressures (SBP) were measured by tail-cuff and then, rats were maintained in metabolic cages during 24 h for urine collections. At euthanasia, remaining kidney was removed for kidney index, histological analyses and renal cortex homogenate preparation. Regardless, salt intake, CTRL rats presented normal SBP, kidney histology, and glomerular filtration rate (GFR). However, CTRL/Salt rats exhibited augmented fractional Na⁺ excretion (FENa), BUN, and proteinuria. DOCA/Salt rats presented exacerbated SBP and FENa, marked kidney injury, and decreased GFR. In all groups, SIK-1 immunofluorescence was localized in mesangial
cells, thick ascending limb, and principal cells. In CTRL rats, high-salt diet increased 40% SIK-1 protein content and 4-fold SIK activity. NKA activity increased from 101±5 to 211±47 nmol Pi×mg⁻¹×min⁻¹ in CTRL/Salt rats, but SIK inhibition brought back NKA to control values. In contrast, in DOCA/Salt rats, SIK-1 protein content did not change, SIK activity increased 2-fold, and NKA activity decreased from 77±5 to 47±6 nmol Pi×mg⁻¹×min⁻¹. In these rats, SIK inhibition significantly decreased NKA activity. Our data indicate that SIK-1 induces Na⁺ reabsorption by increasing NKA activity but during DOCA salt-sensitive hypertension, there is SIK-1 dysregulation, which contributes in part to kidney injury.


Funding: No

Funding Component: P204

Epithelial Sodium Channel Stimulation by Glucose

Parijat S Joy, Peter M. Snyder, Univ of Iowa, Iowa City, IA

There is a link between diabetes mellitus and hypertension, but the underlying mechanisms are poorly understood. The epithelial Na⁺ channel ENaC plays an important role in blood pressure control; ENaC mutations cause Liddle’s syndrome, an inherited form of hypertension. Previous work suggests that ENaC abundance is increased in diabetes mellitus, but the underlying mechanisms are unclear. Here we tested the effect of glucose on ENaC regulation. In Ussing chamber experiments using mouse kidney collecting duct cells (mCCD) and primary cultures of human lung epithelia, elevated glucose increased ENaC-mediated short-circuit current by 2-3 times in a dose-dependent manner from 100mg/dl to 400mg/dl of glucose. This was caused by an increase in ENaC abundance at the cell surface. We hypothesized that hyperglycemia might enhance ENaC cell surface abundance by altering activity of Nedd4-2, an E3 ubiquitin-protein ligase that binds to PY motifs within ENaC. Consistent with this hypothesis, we found that mutation of the PY motifs abolished ENaC stimulation by elevated glucose. Moreover, using a biotinylation assay, we found that elevated glucose (300 mg/dl) slowed ENaC endocytosis and reduced its degradation in the endocytic pathway. These changes in trafficking are explained by our finding that glucose reduced ENaC binding to Nedd4-2, and hence, reduced ENaC ubiquitination. O-GlcNAcylation plays a role in insulin signaling and glucose toxicity due to increased O-GlcNAcylation of target proteins. To test a role for O-GlcNAcylation in ENaC stimulation by glucose, we used 6-Diazo-5-oxo-l-norleucine (DON) to inhibit O-GlcNAcylation. DON abolished ENaC stimulation by elevated glucose. Using anti-O-GlcNAc antibody, we found that Nedd4-2 is a substrate for O-GlcNAcylation, and this modification was increased by elevated glucose. DON also reversed the reduction in binding of Nedd4-2 to ENaC at high glucose levels. Together, our data suggest a model in which hyperglycemia stimulates ENaC through O-GlcNAcylation of Nedd4-2, increasing ENaC abundance at cell surface thus increasing epithelial sodium absorption.

P.S. Joy: A. Employment; Significant; Postdoctoral research fellow. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA 2015 Postdoctoral fellowship. P.M. Snyder: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Research grant from VA.
Abnormal activation of the endogenous renin-angiotensin system (RAS) has been implicated in various cardiovascular (CV) disorders including hypertension, atherosclerosis and stroke. Whereas a low salt diet may be beneficial in salt-sensitive hypertension, it has been proposed to also cause CV risk due to activation of the RAS. The molecular mechanism by which RAS activation mediates vascular dysfunction remains undefined. Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor which activates anti-oxidant and anti-inflammatory processes and can regulate the actions of angiotensin II (AngII) in the vasculature. We examined endothelial function in transgenic mice specifically expressing dominant-negative (DN) mutations in PPARγ in the endothelium (E-V290M) fed a low salt diet to test the hypothesis that endothelial PPARγ plays a protective role in the vasculature in response to endogenous RAS activation. Circulating levels of renin were significantly increased in both non-transgenic (NT) and E-V290M mice fed a low-salt diet for 6 weeks compared to standard chow (NT: 39.3±7.4 vs 19.8±1.3 ng/ml; E-V290M: 34.3±0.8 vs 16.0±3.8 ng/ml, p<0.05, n=5). Under baseline conditions, responses to endothelium-dependent agonist acetylcholine were not affected in E-V290M mice compared to NT (basilar artery: 66.1±11.8 vs 63.5±3.7%; carotid artery: 93.3±3.6 vs 91.1±4.2%, n=5). Six weeks of low-salt diet significantly impaired acetylcholine-mediated dilation in the basilar artery of E-V290M mice but not in NT (41.7±7.7 vs 74.2±5.0%, p<0.05, n=5). Unlike basilar artery, 6 weeks of low salt diet was not sufficient to induce vascular dysfunction in carotid artery or aorta of E-V290M mice (carotid artery: 85.6±4.4 vs 91.9±2.5%, n=5; aorta: 80.8±5.4 vs 87.0±5.6%, n=3). The responses to endothelium-independent vasodilator sodium nitroprusside (SNP) were not different in E-V290M mice compared to NT controls. We conclude that endothelial-specific interference with PPARγ causes endothelial dysfunction in response to endogenous RAS activation induced by a low-salt diet. Moreover, the cerebral circulation is particularly susceptible to low salt diet-induced dysfunction in conjunction with PPARγ impairment.

A.R. Nair: None. M. Mukohda: None. L.N. Agbor: None. C. Hu: None. J. Wu: None. C.D. Sigmund: A. Employment; Significant; University of Iowa. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA SFRN. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Significant; Carver Trust.
Hannah Rosenblum, Columbia Univ Medical Ctr, New York, NY; Stuart Katz, New York Univ Langone Medical Ctr, New York, NY

Background:
Sodium (Na) sensitivity is defined as the short-term increase in blood pressure (BP) after increased Na intake. The impact of Na sensitivity on measures of aortic stiffness has not been previously characterized in non-hypertensives. We tested the feasibility of an oral Na loading protocol to characterize the effects of increased dietary Na on blood pressure and measures of aortic stiffness in healthy young adults.

Methods:
In an open-label repeated measures pilot study, we enrolled 40 healthy adults age 21-30 years (18 females). Subjects were evaluated before and after 7 days of oral Na loading (12g (205 mmol) of salt tablets/day). BP (mmHg, cuff method) was measured in supine, seated and standing positions. Radial artery tonometry (Sphygmacor) was used to assess measures of aortic stiffness: aortic BP (mmHg), aortic pulse pressure (mmHg), aortic augmentation index (%) and adjusted Tr, time of return of the reflected pressure wave as an index of aortic pulse wave velocity (m/s). Serum aldosterone, urine Na and potassium (K) were measured.

Results:
Nine subjects dropped out due to GI intolerance of the salt pills. In the remaining 31 subjects, Na loading did not change BP in any position, aortic BP, aortic pulse pressure, or Tr (Table: mean±SD for seated R arm BP and measures of aortic stiffness). There was no measurable aortic augmentation in the cohort. Na loading decreased serum aldosterone (9.1±1.5 to 5.2±0.8 ng/dl, p=0.02), and increased urine Na:K ratio (1.8±0.2 to 3.5±0.5, p=0.002).

Conclusion:
Oral Na loading was associated with suppression of the renin-angiotensin aldosterone system, did not increase BP, or measures of aortic stiffness in healthy young adults.

<table>
<thead>
<tr>
<th>Brief</th>
<th>SBP</th>
<th>DBP</th>
<th>AldoSys</th>
<th>AldoDiast</th>
<th>AldoPP</th>
<th>AldoTr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>114±11</td>
<td>75±5</td>
<td>10±6</td>
<td>60±7</td>
<td>26±6</td>
<td>1±1</td>
</tr>
<tr>
<td>After</td>
<td>115±15</td>
<td>74±7</td>
<td>9±6</td>
<td>69±7</td>
<td>26±6</td>
<td>1±1</td>
</tr>
</tbody>
</table>

H. Rosenblum: None. S. Katz: None.

Funding: Yes
Funding Component: National Center P207

High Salt Intake desynchronizes the Molecular Clock in Rats

Joshua S Speed, Kelly A Hyndman, Kaehler A Roth, Malgorzata Kasztan, Jermaine G Johnston, Martin E Young, Jennifer S Pollock, David M Pollock, The Univ of Alabama at Birmingham, Birmingham, AL

Circadian rhythms in physiologic functions are driven, at the molecular level, by a group of transcription factors that oscillate over a 24 hour period, collectively termed the molecular clock. Within the kidney, it has been shown that the molecular clock directly influences transcription of Na+ transporters and channels, including ENaC. ENaC is regulated by endothelin-1 (ET-1), via ETβ receptor activation, in response to high salt intake. Thus, we hypothesized that increases in dietary sodium regulate the renal molecular clock (which in turn would facilitate Na+ homeostasis) through an ETβ dependent mechanism. To address this question, we examined the effect of high salt (HS) intake on renal clock gene (Bmal1, Cry1, Per1, Per2) expression. Control and ETβ receptor deficient (ETβ def) rats (a model of elevated renal ENaC) were placed on either HS or normal salt (NS) for two weeks and euthanized every 4 hours beginning at Zeitgeber Time 0 (Lights on). In the inner medulla, HS causes a phase delay in
**Bmal1** (Fig 1A) expression in control but not ETb def rats (Fig 1B). In addition, HS suppressed the expression of **Cry1**, and Per2 during the respective acrophase in both control and ETb def rats (Fig 1C-1F) with no significant effect on Per1. In contrast, no significant difference in the expression of Bmal1, Cry1, Per2, or Per1 (Fig 1I-1P) was found in response to HS in the renal cortex of either control or ETb def. These data indicate that HS feeding desynchronizes the molecular clock within the kidney and provides evidence that peripheral clocks are regulated in a cell type specific manner, even within the same organ.

**Morel E Worou**, Nitin Kumar, Nour-Eddine Rhaleb, Edward L Peterson, Oscar A Carretero, Henry Ford Hosp, Detroit, MI

We recently showed that N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), a natural tetrapeptide with antifibrotic properties prevented high salt-induced albuminuria and renal damage including fibrosis in Dahl Salt-Sensitive (SS) rats and their consomic SS13BN controls. However, the mechanism of this antifibrotic effect is unknown. MicroRNAs (miRNAs) have been shown to be important endogenous regulators of several physiological and pathophysiological conditions. The miRNA let-7 family has been suggested as a negative regulator of profibrotic processes in many diseases and its downregulation has been associated with renal fibrosis. Here, we hypothesized that in SS rats, the antifibrotic effect of Ac-SDKP on high salt-induced renal fibrosis is due to an upregulation of miRNA let-7b expression. Dahl SS and consomic SS13BN rats were fed either 0.23% NaCl (low salt, LS) or 4% NaCl (high salt, HS) diet and infused with vehicle (Veh) or Ac-SDKP (1.6 mg/kg/day) subcutaneously via osmotic minipump for 1 week. Animals were divided into the following groups: LS + Veh, HS + Veh and HS + Ac-SDKP. HS increased the systolic blood pressure (SBP) in SS rats, but not in SS13BN rats. Ac-SDKP did not affect SBP. In both strains, one week of HS diet increased albuminuria. Ac-SDKP prevented HS-induced albuminuria. In SS rats, HS-induced a significant downregulation of the renal cortex miR-let 7b expression measured by quantitative RT-PCR (HS + Veh 0.27±0.16 vs. LS + Veh 1.00±0.09; P=0.005), whereas a treatment with Ac-SDKP significantly upregulated the miR-let 7b expression (HS + Ac-SDKP 2.80±0.11; P=0.002). No miR-let 7b expression difference was observed in SS13BN rats. The overexpression of miR-let 7b by Ac-SDKP is associated with a significant decrease in...
collagen1 mRNA expression (HS + Veh 3.89±0.74 vs. HS + Ac-SDKP 1.25±0.13; P=0.005). One week on HS diet was not sufficient to cause measurable changes in renal collagen protein. In summary, Ac-SDKP prevented albuminuria and renal cortex collagen1 mRNA expression in SS rats fed HS diet for 1 week, and this was associated with an upregulation of miRNA let-7b expression. The antifibrotic effect of Ac-SDKP on HS-induced renal fibrosis in SS rats may be in part due to preceded upregulation of miRNA let-7b expression.

M.E. Worou: None. N. Kumar: None. N. Rhaleb: None. E.L. Peterson: None. O.A. Carretero: None.

Funding: No
Funding Component: P209

Activation of Renal (Pro)Renin Receptor Contributes to High Fuctose-induced Salt Sensitivity

Chuanming Xu, Aihua Lu, Hui Fang, Li Zhou, Sun Yat-sen Univ, Guangzhou, China; Tianxin Yang, Univ of Utah, Salt Lake City, UT

A high-fructose (HF) diet is shown to induce salt-sensitive hypertension but the underlying mechanism remains unclear. The major goal of the present study was to test the role of renal (pro)renin receptor (PRR) in this model. Male Sprague-Dawley rats were randomly divided into the following 4 groups: 1) Control, 2) Fructose, 3) Fructose + PRO20, and 4) Fructose + allopurinol, and the treatments lasted for 3 months. Fructose was added to drinking water (as 20% solution) so was allopurinol (at 30 mg/kg/d). PRO20, an antagonist of (pro)renin receptor, was administered at 700 μg/kg/day via i.p. injections. High fructose (HF) intake induced a 150% increase in renal protein expression of full-length PRR (fPRR), which were attenuated by allopurinol. HF intake also upregulated renal mRNA and protein expression of NHE3 (206% for protein) and NKCC2 (169% for protein) as well as in vivo NKCC2 activity (2-fold increases in 1-h urine volume and UNaV), all of which were nearly completely blocked by PRO20 or allopurinol treatment. HF intake induced >5-fold increases in urinary renin activity, renin content, and total renin content, and a 2-fold increase in urinary AngII, which were suppressed by 60-70% with PRO20 or allopurinol, contrasting to relatively consistent values of these parameters in the plasma, evidence of involvement of intrarenal RAS . At the last week of the experimental period, radio telemetry was performed to monitor blood pressure during one-week high salt (HS) diet (8% NaCl). The 3-mo HF intake or a 1-wk HS diet alone did not affect mean arterial pressure (MAP), but the combination of the two maneuvers induced a ~10 mm Hg increase of MAP, which was abolished by PRO20 or allopurinol treatment. In cultured human kidney 2 cells, both fructose and uric acid (UA) increased protein expression of soluble PRR (sPRR) in a time- and dose-dependent manner; fructose-induced PRR upregulation was inhibited by allopurinol. Taken together, our data suggest that fructose via UA stimulates renal expression of PRR/sPRR that stimulate NHE3 and NKCC2 expression and intrarenal RAS to induce salt-sensitive hypertension.


Funding: No
Funding Component: P210

(Pro)Renin Receptor Regulates Potassium Homeostasis via Intrarenal Aldosterone

Chuanming Xu, Aihua Lu, Hong Wang, Hui Fang, Li Zhou, Peng Sun, Sun Yat-sen Univ,
It has been shown that transgenic overexpression of human (pro)renin receptor (PRR) results in elevated aldosterone (Aldo) level with unclear functional implications. The present study examined a potential role of renal PRR during high K+ (HK) loading. In normal SD rats, a 1-week HK intake (5% KCl in diet) induced a 3.4-fold increase in renal protein expression of full-length PRR and 4.2-fold increase in urinary excretion of soluble PRR (sPRR). Administration of PRO20, a decoy peptide antagonist of PRR, at 700 µg/kg/d via i.p. injections, to K+-loaded animals elevated plasma K+ level (5.72±0.08 vs. 4.84±0.18 mM, p<0.05) and decreased urinary K+ excretion (2.52±0.11 vs. 3.43±0.19 mmol/24h, p<0.05), accompanied with a 26.2% reduction of urinary aldosterone (Aldo) excretion. HK downregulated NCC protein expression (57.8%) and upregulated renal protein expression of aldosterone synthase CYP11B2 (229%), ROMK (156%), calcium-activated potassium channel subunit alpha-1 (α-BK) (367%), α-Na+-K+-ATPase (596%), and β-ENaC (155%), all of which were significantly blunted by PRO20 (by 50 - 70%). The same maneuvers were applied to adrenalectomized (ADX) rats. Although plasma Aldo was extremely low and also unresponsive to HK loading, urinary Aldo excretion was elevated by 274% with this treatment, which was abolished by PRO20 (by 50 - 70%). The same maneuvers were applied to adrenalectomized (ADX) rats. Although plasma Aldo was extremely low and also unresponsive to HK loading, urinary Aldo excretion was elevated by 274% with this treatment, which was abolished by PRO20 (by 50 - 70%). The HK-induced responses of the above K+ and Na+ transporting proteins in ADX rats all persisted and also remained sensitive to PRO20. Additionally, spironolactone treatment in ADX rats was still effective in inhibiting kaliuresis induced by HK loading, resulting in hyperkalemia (Plasma K+: 5.13±0.07 vs. 4.19±0.27 mM, p<0.05). In primary rat IMCD cells, exposure to 10 mM KCl for 24 h augmented PRR protein expression and sPRR release in a time- and dose-dependent manner. HK upregulated Aldo release in parallel with increased CYP11B2 protein expression, which were both attenuated by PRO20 or PRR siRNA. A recombinant sPRR, sPRR-His, stimulated Aldo release and CYP11B2 expression. Taken together, we conclude that HK increased renal PRR expression that stimulates renal synthesis of Aldo that coordinates the response of renal membrane Na+ and K+ transporting proteins to facilitate K+ secretion.


Funding: No

N-Acetyl-Seryl-Aspartyl-Lysyl-Proline (Ac-SDKP) Promotes Tube Formation in Human Coronary Artery Endothelial Cells

Ginette Bordcoch, Pablo Nakagawa, Cesar A Romero, Oscar A Romero, Henry Ford Hosp, Detroit, MI

Ac-SDKP is an endogenous peptide with anti-inflammatory and anti-fibrotic effects in hypertensive and cardiovascular diseases. It is cleaved from Thymosin β4 (Tβ4) and hydrolyzed by angiotensin converting enzyme (ACE). Ac-SDKP plasma concentration increases after treatment with ACE inhibitors (ACEi) and some of the beneficial effects of ACEi treatment has been ascribed to Ac-SDKP. Ac-SDKP is a mediator of angiogenesis in in-vitro and in-vivo animal models. Ac-SDKP stimulates rodents derived immortalized aortic endothelial cells migration and capillary-like structures formation (tube formation). Similarly, Ac-SDKP increases capillary density after myocardial infarction in rats. The mechanism related to angiogenesis induced by Ac-SDKP is not known. Tβ4 (Ac-SDKP precursor) promotes endothelial
cell migration and angiogenesis by the activation of the VEGF/AKT pathway. Our objective is to evaluate the Ac-SDKP pro-angiogenic effect in Human Coronary Artery Endothelial Cells (HCAEC) and the mechanism that regulates the angiogenic effect of Ac-SDKP. HCAEC do not produce VEGF, thus we hypothesize that Ac-SDKP increases VEGF expression in fibroblasts and that indirectly could promote capillary tube formation in endothelial cells. We used primary culture of rat cardiac fibroblast (RCF) and we treated these cells with 10nM Ac-SDKP for 24 hours. VEGF concentration in cell supernatant was measured by ELISA. Cells were starved without serum overnight before the Ac-SDKP treatment. For capillary tube formation assay, HCAEC cells were seeded into matrigel and incubated in presence of 10nM Ac-SDKP for 12 hours, pictures were taken by double phase contrast microscope and tube length was quantified with image J software and the results were expressed as percentage of control. After Ac-SDKP treatment, VEGF concentration did not increase in the supernatant of RCF (control: 0.12±0.07 vs. Ac-SDKP: 0.14±0.09 mg/ml; p=0.7). However, Ac-SDKP treatment induced the development of tube formation in HCAECs by 7±2% respect to control (p=0.037). We conclude that Ac-SDKP induces capillary tube formation not only in rodent but also in human derived endothelial cells. The mechanism by which Ac-SDKP promotes tube formation in HCAECs is still unknown.

G. Bordcoch: None. P. Nakagawa: None. C.A. Romero: None. O.A. Romero: None.

Funding: No

Funding Component: P212

**Sphingolipid De Novo Pathway is a Novel Regulator of Blood Pressure Homeostasis**

Anna Cantalupo, Yi Zhang, Weill Cornell Medical Coll, New York, NY; Xian-Cheng Jiang, SUNY Downstate Medical Ctr, Brooklyn, New York, NY; Annarita Di Lorenzo, Weill Cornell Medical Coll, New York, NY

**Background and objectives:** Sphingolipids, particularly sphingosine 1-phosphate (S1P), play an important role in the cardiovascular homeostasis. Recently, we revealed that endothelial de novo biosynthesis of sphingolipids is very important to control vascular functions and blood pressure. We discovered that in blood vessels, particularly in endothelial cells, Nogo-B, a membrane protein of the endoplasmic reticulum, inhibits serine palmitoyltransferase (SPT), the first and rate-limiting enzyme of de novo production of sphingolipids, to impact vascular tone and blood pressure. Indeed, mice lacking Nogo-B are protected from angiotensin II-induced hypertension, and pharmacological inhibition of SPT by myriocin reinstates high blood pressure in absence of Nogo-B, suggesting that the upregulation of SPT activity exerts anti-hypertensive functions. Thus, the goal of this study is to investigate the role of SPT in vascular functions and blood pressure regulation by using novel genetic mouse models. **Methods:** The SBP was evaluated in 14 weeks old mice heterozygous for Sptlc2 (Sptlc2+/−) or lacking Sptlc2 specifically in endothelial cells (ECKO Sptlc2) and smooth muscle cells (SMCKO Sptlc2) by using tail-cuff system. Vascular reactivity of isolated mesenteric arteries was assessed ex-vivo by using the pressure myograph system. **Results:** Sptlc2+/−, ECKO Sptlc2 and SMCKO Sptlc2 mice were hypertensive compared to their respective controls (Sptlc2+/− 128.9±2.6 vs. WT 112.1±2.6 mmHg; ECKO Sptlc2 125.5±1.8, SMCKO Sptlc2 127.2±0.6 vs. Sptlc2+/− 106±0.84 mmHg) and developed endothelial dysfunction as shown by the impaired vasodilation in response to acetylcholine (EC50 Sptlc2+/− 174.1±7.6 vs. Sptlc2+/− 125.5±1.8).
1.48x10^{-6} \text{ M vs. WT } 4.46x10^{-7} \text{ M; } \text{Emax ECKO } Sptlc2 73.2±3.3\% \text{ vs. } Sptlc2^{f/f} 95.3±1.1\%\), as well as to flow (Emax: Sptlc2^{+/−} 23.3±1.4 \text{ μm vs. WT 42.9±4.4 μm; ECKO Sptlc2 19.9±0.9 μm vs. Sptlc2^{f/f} 41.3±3.1 μm). Conclusion: This study demonstrates the important role of SPT, thus the de novo production of sphingolipids, in controlling blood flow and pressure homeostasis, and provides the ground for the development of alternative therapeutic strategies to manage high blood pressure.

A. Cantalupo: None. Y. Zhang: None. X. Jiang: None. A. Di Lorenzo: None.

Funding: No
Funding Component: P213

Vascular Dysfunction and Aberrant Vascular NOTCH3 Signalling in Hypertension and Cerebral Autosomal Dominant Subcortical Infarcts and Leukoencephalopathy (CADASIL)

Adam Harvey, Fiona Moreton, Augusto C Montezano, Aurelie Nguyen Dinh Cat, Paul Rocchiccioli, Christian Delles, Keith Muir, Rhian M Touyz, Univ of Glasgow, Glasgow, United Kingdom

Hypertension (HT) and CADASIL are clinical conditions of small vessel disease. Vascular dementia is a major feature in CADASIL, and a serious consequence of HT. CADASIL is a monogenic condition due to mutations in NOTCH3, which is expressed almost exclusively in VSMCs. We hypothesised that altered NOTCH3 signalling in CADASIL and HT are associated with small vessel disease. Small arteries from gluteal biopsies from CADASIL patients (n=14), HT patients (n=3) and healthy controls (n=10) were investigated. Vascular function was assessed by myography. Cultured VSMCs were used to assess signaling through NOTCH3, NO, ER stress (gene array) and Rho kinase (ELISA). CADASIL and HT patients exhibited endothelial dysfunction (Max response: CADASIL 41.7±3%, HT 54.1±2% vs Control 98.2±4%). Pre-incubation with N-acetyl-cysteine ameliorated vasorelaxation. Only CADASIL displayed impaired endothelium-independent relaxation (Max response: CADASIL 53±1.9% vs Control 93±8.9%) and contraction (Max response: CADASIL 78±1.3% vs control 102±5%) (p<0.05). AngII-induced contraction was elevated in HT (98%), yet reduced in CADASIL (28%) (vs control 64% max contraction: p<0.05), despite VSMCs from both conditions displaying increased AT_{1}R mRNA expression (HT: 5.1; CADASIL: 3.8; fold vs control; p<0.05). VSMCs from CADASIL and HT have decreased expression of CAMK1, SIRT2 and VEGFA; important in NO signalling (0.5 fold; p<0.05 vs control). VSMC levels of NOTCH3 and NOTCH ligand, JAG1, were increased in CADASIL (3.5, 2.5 fold) and HT (3.0, 2.6 fold, p<0.05). Downstream targets, HEY1 and HEYL, were elevated in CADASIL (3.8, 4.2 fold) and HT (1.9, 2.6 fold) (p<0.05). CADASIL but not HT VSMCs exhibited increased expression of ER stress markers. Rho kinase activity was increased in VSMCs from CADASIL (2.5 fold) and HT (2 fold) vs control (p<0.05). These data demonstrate that in CADASIL and HT, vascular dysfunction, is associated with aberrant NOTCH3 and Rho kinase signalling. In CADASIL, but not HT, endothelium-independent relaxation and ER stress were increased. Our results demonstrate a putative role for NOTCH3 -Rho kinase in vascular dysfunction in conditions of small vessel disease and suggest that ER stress and oxidative stress may be important in vascular injury in CADASIL.


Funding: No
Funding Component: P214
The Non-canonical Stress Tolerance Cascade for Toll-like Receptor 9 in the Vasculature Signals Through Liver Kinase B1

Cameron G. McCarthy, Camilla F. Wenceslau, Safia Ogbi, Theodora Szasz, R.Clinton Webb, Augusta Univ, Augusta, GA

Toll-like receptor (TLR)9 is a pattern recognition receptor of the innate immune system. Recently, a non-canonical stress tolerance pathway has been reported for TLR9 in non-immune cells (cardiomyocytes and neurons), independent of inflammatory signaling. It was observed that TLR9 inhibited sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA)2, increasing cytosolic calcium, and resulting in 5’ AMP-activated protein kinase (AMPK)α activation. In our laboratory, we have reported that TLR9 treatment in vivo causes arterial dysfunction that contributes to the pathogenesis of hypertension and that these phenotypes occurred in conjuction with vascular AMPKα phosphorylation (Thr172). However, whether a dysregulation in calcium homeostasis via the non-canonical stress tolerance cascade underlies the impaired vascular function after TLR9 stimulation needs to be clarified. We hypothesized that TLR9 activation would inhibit SERCA2 activity in the vasculature. SERCA2 activity was assessed using a luciferase-based ATP quantification kit. Microsomes were isolated from pooled aortae of Sprague-Dawley rats and subjected to treatment with either Vehicle (Veh) or ODN2395 (2 μM), with or without a SERCA2 inhibitor (thapsigargin; 1 μM). The presence of thapsigargin increased ATP concentrations similarly in both Veh and ODN2395 [ATP (μM), Veh: 19±3 vs. Veh+thapsigargin: 140±35; ODN2395: 22±9 vs. ODN2395+thapsigargin: 129±12, both p<0.05], suggesting TLR9 activation did not inhibit SERCA2 activity. Next, MRA from Sprague-Dawley rats were divided into three sections for Western blot analysis of AMPKα-activating kinases, specifically calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) and liver kinase B1 (LKB1). ODN2395 alone did not increase protein expression of phospho-CaMKK2Ser511 (p>0.05), again suggesting calcium-independent activation of AMPKα. However, ODN2395 did increase phospho-LKB1Ser428 (3.8 fold vs. Veh, p<0.05), and this increase in expression was inhibited by pre-incubation with TLR9 antagonist ODN2088 (20 μM) (p>0.05). These results suggest that the TLR9 non-canonical stress tolerance pathway in the vasculature is mediated by LKB1, and not SERCA2 inhibition.


Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P215

Nonalcoholic Fatty Liver Disease is Associated with Coronary Artery Calcification: a Systematic Review and Meta-analysis

Veeravich Jaruvongvanich, Univ of Hawaii, Honolulu, HI; Anawin Sanguankeo, Bassett Medical Ctr, Cooperstown, NY; Kamonkiat Wirunsawanya, Univ of Hawaii, Honolulu, HI; Sikarin Upala, Bassett Medical Ctr, Cooperstown, NY

Background: Whether nonalcoholic fatty liver disease (NAFLD) is related to subclinical atherosclerosis is unclear. Coronary artery calcium scanning (CAC) is the robust predictor of coronary events in the asymptomatic individuals. Several recent studies have investigated the association between NAFLD and this surrogate marker. Thus, we conducted a systematic review and meta-analysis to better
characterize the association between NAFLD and CAC. **Methods:** A comprehensive search of the databases of the MEDLINE and EMBASE was performed from inception through May 2016. The inclusion criterion was the observational studies’ assessment of the association between NAFLD and CAC in adult subjects. Pooled odds ratio (OR) and 95% confidence interval (CI) from multivariate model with confounders adjustment were calculated using a random-effect, generic inverse variance method. The between-study heterogeneity of effect-size was quantified using the $Q$ statistic and $I^2$. **Results:** Data were extracted from 15 studies (all cross-sectional studies) involving 35,409 subjects. NAFLD is significantly associated with CAC > 0 and CAC > 100 with pooled OR of 1.41 (95% CI 1.24-1.61, $P_{\text{heterogeneity}} = 0.01, I^2=57\%$) (Figure 1) and 1.24 (95% CI 1.02-1.52, $P_{\text{heterogeneity}} = 0.09, I^2=42\%$). The association between NAFLD and CAC > 0 was stronger in women than men ($P_{\text{between sex}} = 0.017$). **Conclusions:** NAFLD is also associated with coronary calcification independent of traditional risk factors, obesity and metabolic syndrome in asymptomatic individuals and this association appeared to be stronger in women.

**Association Between White-coat Hypertension and Arterial Stiffness: a Systematic Review and Meta-analysis**

Sikarin Upala, Anawin Sanguankeo, Bassett Medical Ctr, Cooperstown, NY

**Background:** Previous studies have shown inconclusive effects of target organ damage from white-coat hypertension (WCHT). Arterial stiffness is involved in the atherosclerotic processes in the setting of sustained hypertension. This meta-analysis aimed to compare arterial stiffness in subjects with diagnosis of WCHT to subjects with normotension (NT) and SHT. **Methods:** A comprehensive search of the databases of the MEDLINE and EMBASE was performed from inception through May 2016. The inclusion criterion was the observational studies’ assessment of the association between WCHT and NT or SHT in adult subjects. European Society of Hypertension practice guidelines for ambulatory blood pressure (BP) monitoring was used to define WCHT (office BP≥140/90mmHg and daytime BP <135/85mmHg), and SHT (office BP≥140/90mmHg and daytime BP≥135/85mmHg). Aortic stiffness was assessed using Carotid-femoral pulse wave velocity (PWV) measurements. Pooled mean difference (MD) of PWV and 95% confidence interval (CI) were calculated using a random-effect, generic inverse variance method. **Results:** Data were extracted from 4 observational studies involving 2,413 subjects. PWV is not different in patients with WCHT compared with SHT (pooled MD= -0.25 m/sec; 95% CI, -0.81 to 0.30; $P$-value=0.37, $I^2=74\%$). PWV in WCHT is also not different when compared with PWV in NT (MD=0.86 m/sec; 95% CI, -0.30 to 2.03; $P$-value=0.15, $I^2=97\%$). **Conclusion:** In a meta-analysis, we observe that arterial stiffness measured by pulse wave velocity is not different in patients

<table>
<thead>
<tr>
<th>Study name</th>
<th>Odds ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Relative weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juarez-Rojas 2013</td>
<td>2.340</td>
<td>1.070</td>
<td>5.119</td>
<td>2.48</td>
</tr>
<tr>
<td>Kim 2012</td>
<td>1.340</td>
<td>1.238</td>
<td>1.562</td>
<td>17.31</td>
</tr>
<tr>
<td>Kim 2015</td>
<td>1.488</td>
<td>0.962</td>
<td>2.301</td>
<td>6.50</td>
</tr>
<tr>
<td>Mellinger 2015</td>
<td>1.170</td>
<td>1.075</td>
<td>1.274</td>
<td>21.59</td>
</tr>
<tr>
<td>Ono 2015</td>
<td>1.950</td>
<td>0.942</td>
<td>4.038</td>
<td>2.82</td>
</tr>
<tr>
<td>Posados-Ramones 2014</td>
<td>3.050</td>
<td>1.399</td>
<td>7.356</td>
<td>1.80</td>
</tr>
<tr>
<td>Rhee 2015</td>
<td>1.370</td>
<td>1.114</td>
<td>1.689</td>
<td>14.84</td>
</tr>
<tr>
<td>Sung 2012</td>
<td>1.210</td>
<td>1.010</td>
<td>1.450</td>
<td>16.39</td>
</tr>
<tr>
<td>Sung 2013</td>
<td>1.953</td>
<td>1.450</td>
<td>2.631</td>
<td>10.59</td>
</tr>
<tr>
<td>You 2015</td>
<td>1.550</td>
<td>0.966</td>
<td>2.486</td>
<td>5.76</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1.411</strong></td>
<td><strong>1.239</strong></td>
<td><strong>1.607</strong></td>
<td><strong>2.48</strong></td>
</tr>
</tbody>
</table>

V. Jaruvongvanich: None. A. Sanguankeo: None. K. Wirunsawanya: None. S. Upala: None.

Funding: No

Funding Component: P216
with white-coat hypertension when compared with sustained hypertension or normotension.

S. Upala: None. A. Sanguankeo: None.

Funding: No
Funding Component: P217

Mitochondrial N-Formyl Peptides Elicit Changes in Endothelial Cell Cytoskeleton via Formyl Peptide Receptor Activation

Camilla F Wenceslau, Cameron G. McCarthy, R.Clinton Webb, Augusta Univ, Augusta, GA

One major pathophysiological characteristic of cardiovascular disease, including hypertension, is vascular dysfunction. Recently, we demonstrated that mitochondrial damage-associated molecular patterns are elevated in the circulation of SHR. Mitochondria carry hallmarks of their bacterial ancestry and one of these hallmarks is that this organelle still uses an N-formyl-methionyl-tRNA as an initiator of protein synthesis. We observed that mitochondrial N-formyl peptides (F-MIT) infusion into rats induces inflammation and vascular dysfunction, including vascular leakage, via formyl peptide receptor (FPR) activation. However, neutrophil depletion did not change this response. Therefore, we hypothesize that F-MIT via FPR activation elicits changes directly to cytoskeleton-regulating proteins in vascular cells, which may lead to increased vascular permeability. To test this hypothesis we used vascular smooth muscle cells (VSMC) and endothelial cells harvested from aortas of Sprague-Dawley rats (n=5) and human donors (n=5), respectively. Cells were divided into three groups for Western blot analysis of cytoskeleton-regulating proteins. The cells were incubated for 20 minutes in medium with either vehicle (non-formylated peptide), F-MIT (10 μM), or F-MIT after a 5-minute pre-incubation with FPR1 and 2 antagonists (Cyclosporine H, CsH, 1 μM and WRW4, 10 μM). In endothelial cells, the treatment with F-MIT increased the expression of RhoA/ROCK (Rho: 1.8 fold vs. Veh; ROCK: 1.4 fold vs. Veh, p<0.05), cell division control protein 42 (CDC42) (2.0 fold vs. Veh, p<0.05) and phospho-myosin light chain (MLC)Thr/Ser19 (1.5 fold vs. Veh, p<0.05). These changes were all abolished in the presence of FPR antagonists. On the other hand, F-MIT decreased expression of phospho-MLC (0.6 fold vs. Veh, p<0.05) and CDC42 (0.5 fold vs. Veh, p<0.05) and did not change RhoA/ROCK expression in VSMC. In conclusion, F-MIT, via FPR activation, elicits direct changes in endothelial cell and VSMC cytoskeleton-regulating proteins. This interaction can lead to endothelial contraction, increased vascular leakage and attenuated barrier function as observed in clinical and experimental hypertension.

C.F. Wenceslau: None. C.G. McCarthy: None. R. Webb: None.

Funding: Yes
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P218

Induction of Human Endothelin-1 Overexpression for 3 Months Causes Blood Pressure Rise and Small Artery Endothelial Dysfunction and Stiffening

Suellen C Coelho, Sofiane Ouerd, Julio C. Fraulob-Aquino, Hypertension and Vascular Res
Unit, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Tili Barhoumi, Hypertension and Vascular Res Unit, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Stefan Offermanns, Dept of Pharmacology, Max-Planck-Inst for Heart and Lung Res, Bad Nauheim, Germany; Pierre Paradis, Hypertension and Vascular Res Unit, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada, Montreal, QC, Canada; Ernesto L Schiffrin, Hypertension and Vascular Res Unit, Lady Davis Inst for Medical Res and Dept of Med, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada

Background: The mechanisms of blood pressure (BP) regulation by endothelin (ET)-1 produced by endothelial cells are complex and remain unclear. Recently, we developed a transgenic mouse with tamoxifen-inducible endothelium-restricted human ET-1 overexpression (ieET-1) using Cre/loxP technology. ieET-1 mice exhibited BP rise after three weeks of induction in an ET type A (ETA) receptor-dependent manner, in absence of vascular and kidney injury. It is unknown whether long-term exposure to ET-1 overexpression results in sustained BP elevation and vascular injury.

Design and methods: Nine to 12-week old male ieET-1 mice and control ieCre mice expressing a tamoxifen-inducible Cre recombinase under the control of endothelium-specific Tie2 promoter, were treated with tamoxifen (1 mg/kg/day, s.c.) for 5 days and studied 3 months later. ieET-1 mice were treated or not with ETA receptor blocker, atrasentan (10 mg/kg/day, PO) in the last 2 weeks of the study. BP by telemetry, endothelial function and vascular remodeling by pressurized myography and reactive oxygen species (ROS) generation using dihydroethidium staining in mesenteric artery (MA) or perivascular fat (PVAT) and renal artery flow (RAF) by ultrasonography were determined.

Results: Systolic BP was increased in ieET-1 and normalized by atrasentan compared to ieCre mice (141±0 and 124±4 vs 120±0 mm Hg, P<0.001). Endothelium-dependent relaxation responses to acetylcholine were impaired in ieET-1 and uncorrected by atrasentan compared to ieCre (35±4 and 32±4 vs 65±8%, P<0.01). Media/lumen and media cross-sectional area were unchanged, but stiffness was increased in ieET-1 and normalized by atrasentan compared to ieCre mice (strain at 140 mm Hg: 0.6±0.0 and 0.7±0.0 vs 0.7±0.0 ΔD/D, P<0.05). ROS generation was enhanced in PVAT of ieET-1 and normalized with atrasentan when compared to ieCre mice (1.4±0.1 and 0.9±0.1 and vs 1.0±0.1 relative fluorescence units/µm², P<0.05). RAF was decreased in ieET-1 and unchanged by atrasentan compared with control (1.8±0.2 and 2.0±0.2 vs 3.0±0.3 mL/min, P<0.01).

Conclusions: Long-term exposure to endothelial human ET-1 overexpression caused sustained BP elevation, endothelial dysfunction and vascular stiffening and oxidative stress.


Funding: No

Exercise Training Normalizes Vascular Changes in Aging Hypertension Involving microRNAs Profile and Target Genes

Tiago Fernandes, Fernanda Roberta Roque, Vander José das Neves, João L. Penteado, André C. Silveira, Suliana Mesquita, Camila P. Jordão, Rodrigo Souza, Luciana V. Rossoni, Edilamar Menezes de Oliveira, Univ of Sao Paulo, Sao Paulo, Brazil
MiRNAs profile and target genes involved in hypertension-associated vascular changes were evaluated. In addition, we checked the therapeutic role of exercise training (ET) on these parameters. Spontaneously hypertensive rats (SHR) aged 6 months and their controls Wistar Kyoto (WKY) were divided into 4 groups: SHR, trained SHR (SHR-T), WKY and trained WKY (WKY-T). Swimming ET consisted of 60 min of duration, 1x/day/10 weeks, with 4% caudal body weight workload. SHR showed an increased in systolic blood pressure (207 ± 5.5 mmHg) compared to WKY (133 ± 3.9 mmHg) analyzed by tail-cuff system, with no changes in baseline heart rate. We observed a reduction in VO2 peak (WKY: 62 ± 1.5; SHR: 53 ± 2.5 mL.kg⁻¹.min⁻¹) accompanied by soleus muscle atrophy (fiber type I - WKY: 4039 ± 195; SHR: 2658 ± 53; type IIa - WKY: 2903 ± 182, SHR: 2050 ± 68; Intermediate - WKY: 2663 ± 136, SHR: 1967 ± 95 µm²) in SHR. Vascular function of femoral artery was similar in SHR and WKY, however, wall-to-lumen ratio was increased in femoral artery (WKY: 0.17±0.01, SHR: 0.27±0.01 a.u.) and muscular arteriole (WKY: 0.54±0.02, SHR: 1.14±0.03 a.u.) accompanied by capillary rarefaction (WKY: 1.2 ± 0.05, SHR: 0.6 ± 0.03 capillary-to-fiber ratio) in soleus muscle from SHR vs. WKY. In contrast, ET promoted reduction in blood pressure and resting bradycardia in trained animals. ET corrected the VO2 peak reduction, muscle wasting, microvascular remodeling in SHR-T toward control levels. ET downregulated 8 miRNAs (-96, -205, -182, -146b-5p, -140, -328a, -665, -1) and upregulated 3 miRNAs (-499, -208b and -99b) in SHR-T when compared to SHR. Bioinformatics study for functional analysis of the predicted targets genes for the 11 miRNAs restored by ET demonstrated enrichment of different signaling pathways including cell death (p value <0.001; Fold enrichment 16.78) and vascular development (p value < 0.001; Fold enrichment 10.62). Thus, targets involved in angiogenesis and vascular integrity by the VEGF/VEGFR2/AKT/eNOS/Bcl-2 pathway were impaired in hypertension and corrected by ET. The results support the hypothesis that the structural changes arising from the progression of hypertension may be regulated by a set of miRNAs and target genes; and ET participates in restoring the vascular remodeling.

Funding: No
Funding Component: P220

Matrix Metalloproteinase 2 Knockout Protects from Angiotensin II-induced Vascular Injury

Julio C. Fraulob-Aquino, Tili Barhoumi, Muhammad O Mian, Nourreddine Idris-Khodja, Ku-Geng Huo, Asia Rehman, Talin Ebrahimian, Stéphanie Lehoux, Pierre Paradis, Vascular and Hypertension Res Unit, Lady Davis Inst for Medical Res, McGill Univ, Montreal, QC, Canada; Ernesto L Schiffrin, Vascular and Hypertension Res Unit, Lady Davis Inst for Medical Res and Dept of Med, Sir Mortimer B. Davis-Jewish General Hosp, McGill Univ, Montreal, QC, Canada

Objective: Matrix metalloproteinase-2 (MMP2) participates in the mechanisms of vascular injury in atherosclerosis. Whether MMP2 plays a role in angiotensin (Ang) II-induced hypertension and vascular remodeling is unknown. We hypothesized that Mmp2 knockout (Mmp2⁻/⁻) would prevent Ang II-induced hypertension and vascular injury.

Methods and Results: Fourteen days of Ang II infusion (1000 ng/kg/min, SC) increased systolic blood pressure (SBP, 161±9 vs 122±3 mm Hg, P<0.05) and decreased vasodilatory responses
to acetylcholine (33±5 vs 83±3%, P<0.001), increased the media/lumen (4.8±0.4 vs 3.1±0.1%, P<0.001) and media cross-sectional area (7223±467 vs 5346±336 µm², P<0.05) and enhanced stiffness (P<0.05), as shown by a leftward shift of the stress/strain relationship of mesenteric arteries in wild-type mice. Ang II enhanced aortic (73±6 vs 6±1 relative fluorescence units [RFU]/µm², P<0.001) and perivascular adipose tissue (PVAT) reactive oxygen species generation (76±11 vs 12±1 RFU/µm², P<0.001), aortic VCAM-1 (17±3 vs 5±1 RFU/µm², P<0.001) and MCP-1 expression (71±14 vs 11±3 RFU/µm², P<0.001), PVAT monocyte/macrophage (1.8±0.3 vs 0.1±0.1 % of PVAT, P<0.001) and T cell infiltration (56±14 vs 16±9 cells/µm², P<0.05) and the fraction of spleen activated CD4+CD69+ (17±2 vs 10±1 % of CD4+ T cells, P<0.001), CD8+CD69+ T cells (11±1 vs 5±1 % of CD4+ T cells, P<0.001) and Ly-6Chi monocytes (53±6 vs 25±2 % event, P<0.001). Ang II increased phosphorylation of epidermal growth factor receptor 1.9±0.2-fold and extracellular-signal-regulated kinase 1/2 1.4±0.1-fold in vascular smooth muscle cells isolated from mesenteric arteries of wild-type mice (P<0.05). Mmp2 knockout prevented or reduced all of the above (P<0.05) except SBP elevation. Bone marrow transplantation experiments revealed that Ang II-induced hypertension was impaired in absence of immune cell MMP2 and endothelial dysfunction was blunted or reduced in absence of immune or vascular cell MMP2 (P<0.05).

**Conclusions:** Mmp2 knockout prevented Ang II-induced vascular injury but not hypertension. Bone marrow transplantation experiments revealed a complex relationship of immune and vascular cell MMP2 in the development of Ang II-induced hypertension and endothelial dysfunction.


**Funding:** No

**Funding Component:** P221

**Vascular Smooth Muscle-Specific Overexpression of Cyp4a12-20-HETE Synthase Causes Hypertension and Vascular Remodeling**

Rebecca Hutcheson, Frank Zhang, Katherine Gottlinger, Michal Schwartzman, New York Medical Coll, Valhalla, NY

20-Hydroxyeicosatetraenoic acid (20-HETE) is a potent vasoactive eicosanoid of the microcirculation exhibiting effects on vascular smooth muscle (VSM) function that include stimulation of contractility, migration and growth. We have previously demonstrated in mice which globally overexpress 20-HETE that hypertension as well as vascular remodeling is not fully prevented by pharmacological normalization of blood pressure. However, both hypertension and vascular remodeling are prevented by antagonism of 20-HETE, suggesting that 20-HETE exerts vascular effects independent of hypertension. To examine the contribution of VSM-derived 20-HETE to hypertension and vascular remodeling, we have developed a transgenic mouse model that overexpresses the primary mouse 20-HETE synthase, Cyp4a12, specifically in VSM by crossbreeding floxed Cyp4a12 with Myh11-Cre mice. All mice carry the floxed Cyp4a12 gene and express a GFP protein under control of a modified CMV promoter. Cre-recombination excises the GFP cDNA and inserts the Cyp4a12 cDNA.

Three to four month old male Cyp4a12-floxed mice carrying the Myh11-Cre gene (Cyp4a12fl/flMyh11Cre+/−) exhibited higher systolic blood pressure than control mice Cyp4a12fl/flMyh11Cre−/− (WT) mice (135 vs. 115 mmHg, n=4, *p<0.05). 20-HETE production in
Cyp4a12fl/flMyh11Cre+/- mice were higher than in WT in mesenteric (3333±891 vs. 545±196 ng/mg/h) and renal interlobar arteries (RIA; 1859±376 vs. 242±62 ng/mg/h). Plasma levels of 20-HETE were also elevated (324±61 vs. 185±34 pg/mL) while urinary levels were not significantly different (146±26 vs. 117±29 pg/mL). We also observed increased medial thickness and decreased lumen area of blood vessels by Myh11 immunofluorescence in heart and kidney sections. RIA from male Myh11-Cyp4a12 mice displayed higher constrictor sensitivity to phenylephrine and impaired relaxation to acetylcholine compared to WT. Taken together, these data suggest that this model displays hypertension and pathological hypertrophic vascular remodeling. We therefore conclude that the Cyp4a12fl/flMyh11Cre+/- is a promising and unique transgenic mouse model to examine the contribution of smooth muscle-derived 20-HETE to hypertension and hypertrophic vascular remodeling.

BACKGROUND A significant number of studies have shown a positive correlation between Hcy plasma levels and hypertension. On the other hand, pathogen recognition receptors, and in particular TLR-4 is a foreign antigen sensor that plays role in innate immune system activation and has recently gained a significant attention in the field of hypertension. Mitochondrial dysfunction and mitochondria-dependent apoptosis have been shown to promote endothelial cell loss leading to endothelial dysfunction that contributes to pathogenesis of hypertension. These events induce mitochondrial dysfunction characterized by excessive mitochondrial fission and mitochondrial apoptosis contributing to vascular remodeling followed by hypertension.

OBJECTIVE The objective of this study is to define the mechanisms of homocysteine effect on aortic wall that promote vascular remodeling and hypertension and explore the role of TLR-4 mutation in alleviation of homocysteine negative effects.

METHODS For this study we used 5 groups of mice: C57BL/6J, C3H/HEouJ, CBS+/-; C3H/HeJ, and CBS+/-/C3H. For further analysis we used isolated aorta and collected blood. Blood pressure was recorded using noninvasive tail cuff method. Effects of hyperpolarization factor and endothelial-dependent vasodilator on aorta contractility were also performed. We checked expression of mitochondrial fusion and fission proteins, antioxidant markers and expression of collagen/elastin fragments.

RESULTS Data showed that there were increased values of systolic and diastolic pressure in CBS+/- mice (DP: 127.06±18.24; SP: 159.59±15.84) and C3H/HeJ mice had decreased levels of in comparing to other groups (DP: 55.43±16.19; SP: 110.04±5.90). The response to hyperpolarization factor and endothelial-dependent vasodilator were blended in CBS+/- aorta, however mitigated in CBS+/-/C3H. Fusion and fission ratio (Mfn2/DRP1) were increased in C3H/HeJ mice (1.53±0.08) and mostly decreased in CBS+/- mice (0.42±0.05) comparing to other groups.

CONCLUSION These findings indicate the prevalence of mitochondrial fission over mitochondrial fusion in HHcy may explain possible endothelial cell loss and dysfunction.
followed by collagen accumulation that contributes to vascular remodeling.


Funding: No
Funding Component: P223

Caveolin-1 Contributes to Vascular Remodeling and Inflammation Induced by Angiotensin II in vivo and in vitro

Tatsuo Kawai, Steven J Forrester, Katherine J Elliot, Takashi Obama, Takehiko Takayanagi, Kevin Crawford, Satoru Eguchi, Victor Rizzo, Temple Univ Sch of Med, Philadelphia, PA

We have recently reported that caveolin-1 (Cav1) enriched membrane microdomains in vascular smooth muscle cells (VSMC) mediate a metalloprotease ADAM17-dependent EGF receptor (EGFR) transactivation, which is linked to vascular remodeling but not contraction induced by angiotensin II (AngII). We have tested our hypothesis that Cav1, a major structural protein of caveolae, plays a critical role for development of vascular remodeling but not hypertension induced by AngII. 8 week old male Cav1-/- and the control Cav+/+ wild-type mice (WT: C57BL6) were infused with AngII (1 μg/kg/min) for 2 weeks to induce vascular remodeling and hypertension. Upon AngII infusion, histological assessments demonstrated medial hypertrophy and perivascular fibrosis of coronary and renal arteries in WT mice compared with sham-operated control mice. The AngII-infused WT mice also showed a phenotype of cardiac hypertrophy with increased heart weight/body weight (HW/BW) ratio (8.6±0.5 vs 6.4±0.2 p<0.05). Similar levels of AngII-induced hypertension were observed in both WT and Cav1-/- mice assessed by telemetry (mean arterial pressure: 142±9 vs 154±20 mmHg). In WT mice, Ang II enhanced ADAM17 expression and phospho-Tyr1068 EGFR staining in vasculatures of heart and kidney. These events were attenuated in vessels from Cav1-/- mice infused with AngII. In addition, immuno-histochemical analyses revealed less ER stress in heart and kidney of AngII-infused Cav1-/- mice compared with WT mice. Enhanced Cav1 and VCAM-1 expression were also observed in aorta from AngII-infused WT mice but not in Cav1-/- aorta. In rat VSMCs, adenoviral encoding Cav1 siRNA (100 moi) attenuated AngII-induced enhancements of total cell protein, cell volume and extracellular collagen content but not mitochondrial ROS generation. These data suggest that Cav1 and presumably vascular caveolae play critical roles for vascular remodeling and inflammation which likely involves the ADAM17/EGFR cascade independent from blood pressure or mitochondrial ROS regulation.


Funding: Yes
Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

P224

Endothelin-1 Overexpression Preserves Endothelial Function in Mice with Vascular Smooth Muscle Cell-specific Deletion of PPAR-gamma

Sofiane Ouerd, Noureddine Idris-Khodja, Hypertension and Vascular Res Unit, Lady Davis
Objective: Peroxisome proliferator-activated receptor γ (PPARγ) agonists reduce blood pressure (BP) and vascular injury in hypertensive rodents. Pparγ inactivation in vascular smooth muscle cells (VSMC) using a tamoxifen inducible Cre-Lox system enhanced angiotensin II-induced vascular damage. Transgenic mice overexpressing endothelin (ET)-1 in the endothelium (eET-1) exhibit endothelial dysfunction, increased oxidative stress and inflammation. We hypothesized that inactivation of the Ppar gene in VSMC (smPparγ⁻/⁻) would exaggerate ET-1-induced vascular damage.

Methods and Results: Eleven-week-old male control, eET-1, smPparγ⁻/⁻ and eET-1/smPparγ⁻/⁻ mice were treated with tamoxifen (1 mg/kg/day, s.c.) for 5 days and sacrificed 4 weeks later. Systolic BP determined by telemetry was higher in eET-1 (123±5 vs 109±2 mm Hg, P<0.05) and unaffected by smPparγ inactivation. Mesenteric artery (MA) vasorelaxation to acetylcholine was impaired only in smPparγ⁻/⁻ (Emax: 52.0±6.7 vs 82.2±4.9%, P<0.05). MA reactive oxygen species levels were increased 1.7±0.3-fold in smPparγ⁻/⁻ (P<0.05) and further increased in eET-1/smPparγ⁻/⁻ (2.5±0.3-fold, P<0.05). MA perivascular fat monocyte/macrophage infiltration was higher in eET-1 and smPparγ⁻/⁻ (331±34 and 326±49 vs 140±8 cells/mm², P<0.05), and further increased in eET-1/smPparγ⁻/⁻ (557±77, P<0.05). The spleen fraction of CD11b⁺ cells was increased in smPparγ⁻/⁻ (1.1±0.1 vs 0.47±0.1%, P<0.05) and further increased in eET-1/smPparγ⁻/⁻ (1.8±0.2%, P<0.05). The spleen fraction of Ly-6Chi monocytes was increased in eET-1 and smPparγ⁻/⁻ (24±3 and 27±4 vs 14±1%, P<0.05) but not in eET-1/smPparγ⁻/⁻. The spleen fraction of T regulatory cells was increased in smPparγ⁻/⁻ (13±2 vs 9±1%, P<0.05) and decreased in eET-1 (7±1%, P<0.05), which was further decreased by eET-1/smPparγ⁻/⁻ (3±1%, P<0.05).

Conclusions: These results suggest that increased ET-1 paradoxically preserves endothelial function in mice with inactivated VSMC Ppar despite enhanced oxidative stress. Flow cytometry data indicate that infiltrating monocyte/macrophages in these mice might be anti-inflammatory.


Funding: No

Funding Component: P225

Nox1 or Nox4 Deletion Prevents Type-1 Diabetes-induced Endothelial Dysfunction

Sofiane Ouerd, Noureddine Idris-Khodja, Hypertension and Vascular Res Unit, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada;
Objective: The prognosis of type-1 diabetes is in part related to the increased risk of vascular complications such as atherosclerosis. Overproduction of reactive oxygen species by NADPH oxidase (NOX) is believed to play an important role in diabetes-related vascular injury. NOX1 may play a role in the macrovascular disease, whereas NOX4 may have protective actions. Nevertheless, their role in diabetic vascular injury is less well understood. We hypothesized that deletion of Nox1 would prevent diabetes-induced endothelial dysfunction and vascular remodeling of small arteries whereas Nox4 would exaggerate vascular injury in atherosclerosis-prone apolipoprotein knockout (Apoep+/−) mice.

Methods: Diabetes was induced by streptozotocin IP injections (STZ, 55 mg/kg/day) for 5 days in 6-week-old male Apoe−/− mice, Apoe−/− mice deficient in Nox1 (Apoep−/−/Nox1−/−) and Nox4 (Apoep−/−/Nox4−/−). Mice were studied 14 weeks later. Endothelial function and vascular remodeling were assessed in mesenteric arteries (MA) using pressurized myography.

Results: Apoe−/− mice presented a maximal endothelium-dependent vasodilatory response (Emax) to acetylcholine of 48±8%, which was further decreased by diabetes to 20±6%. In contrast, endothelium-dependent relaxations to acetylcholine were 1.5-fold higher in diabetic Apoe−/−/Nox1−/− and Apoe−/−/Nox4−/− mice compared to non-diabetic Apoe−/− mice (Emax: 72±9 and 70±7 vs 48±8%). Diabetes decreased MA stiffness in Apoe−/− mice, as indicated by a rightward displacement of the stress-strain curves (strain at 140 mm Hg: 1.02±0.04 vs 0.75±0.04), which was blunted by Nox1 or Nox4 knockout (strain at 140 mm Hg: 0.77±0.02 and 0.81±0.02). MA media/lumen was unaltered by diabetes. Knockout of Nox4 but not Nox1 increased MA media/lumen 1.4-fold in diabetic Apoe−/− mice (4.1±0.4 and 3.5±0.2 vs 2.9±0.2%).

Conclusions: These results suggest that NOX1 and NOX4 play a pathophysiological role in diabetes-induced endothelial dysfunction and contribute to potentially maladaptive changes in vascular stiffness. NOX4 seems to have dual actions on the vasculature, as it is also protective against vascular remodeling of small arteries in type 1 diabetes.


Funding: No

Funding Component: P226

Endothelin-1 Overexpression Exaggerates Diabetes-induced Endothelial Dysfunction by Altering Oxidative Stress

Objective: Increased endothelin (ET)-1 expression has been shown to cause endothelial dysfunction and oxidative stress. Plasma ET-1 is increased in patients with diabetes mellitus. Since endothelial dysfunction often precedes vascular complications in diabetes, we sought to determine whether ET-1 contributes to diabetes-induced endothelial dysfunction. We hypothesized that overexpression of ET-1 in the endothelium will exaggerate diabetes-induced endothelial dysfunction.

Methods: Diabetes was induced by streptozotocin IP injections (STZ, 55 mg/kg/day) for 5 days in 6-week-old male wild-type (WT) mice and in mice overexpressing human ET-1 restricted to the endothelium (eET-1). Mice were studied 14 weeks later. Endothelial function and vascular remodeling using pressurized myography, reactive oxygen species (ROS) production by dihydroethidium staining and mRNA expression by reverse transcription-quantitative PCR were assessed in mesenteric arteries (MA).

Results: MA endothelium-dependent vasodilatory responses to acetylcholine were reduced 24% by diabetes in WT (Emax: 61±6 vs 84±3%, P<0.05), and further decreased by 12% in eET-1 (Emax: 49±5, P<0.05). Diabetes decreased MA media/lumen in WT (2.4±0.1% vs 3.3±0.2%, P<0.05) and eET-1 (2.9±0.2% vs 4.0±0.2%, P<0.05), whereas ET-1 overexpression increased MA media/lumen to a similar extent in diabetic and non-diabetic WT mice (P<0.05). Vascular ROS production in MA was increased 2-fold by diabetes in WT (5.0±0.5 vs 2.5±0.3 relative fluorescence units [RFU]/µm², P<0.05) and further augmented 1.7-fold in eET-1 (8.5±1.2 RFU/µm², P<0.05). Diabetes reduced endothelial nitric oxide synthase (eNOS, Nos3) mRNA expression in eET-1 by 50% (0.7±0.1 vs 1.4±0.2, P<0.05) but not in WT. Induction of diabetes caused a 50% increase in superoxide dismutase 1 (Sod1, 1.5±0.2 vs 1.0±0.0, P<0.05) and a 30% increase in Sod2 (1.3±0.1 vs 1.0±0.0, P<0.05) mRNA expression in WT but not in eET-1.

Conclusions: Increased expression of ET-1 exaggerates diabetes-induced endothelial dysfunction. This may be caused by an increase in vascular oxidative stress, a decrease in eNOS expression and a decrease in antioxidant capacity.


Funding: No

Funding Component: P227

Mapping of Chromosome 2 Regions Linked to Vascular Inflammation Using Congenic Rats

Sofiane Ouerd, Noureddine Idris-Khodja, Suellen C. Coelho, Antoine Caillon, Olga Berillo, Hypertension and Vascular Res Unit, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Anne E. Kwitek, Dept of Internal Med, Univ of Iowa, Iowa, IA; Pierre Paradis, Hypertension and Vascular Res Unit, Lady Davis Inst for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Ernesto L. Schiffrin, Hypertension and Vascular Res Unit, Lady Davis Inst for Medical Res, Dept of Med, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada

Objective: Chromosome 2 (chr2) introgression from normotensive Brown Norway rats (BN) into hypertensive Dahl salt sensitive (SS) background (consomic SS2BN) reduced blood pressure (BP) and vascular inflammation under a normal-salt diet. Mapping of chr2 using congenic rats revealed that the distal portion of
BN chr2 (SS2BNα) but not the middle segment (SS2BNb) on the SS background under a normal-salt diet contains anti-inflammatory genes. However, the role of chr2 in the regulation of vascular inflammation under a high-salt diet (HSD) remains unknown. We hypothesized that SS2BNα but not SS2BNb rats would have reduced vascular inflammation under HSD.

**Design and method:** Four-to-6 week old male SS, SS2BNα and SS2BNb rats were fed a HSD (4% NaCl) for 8 weeks or until they developed a stroke as manifested by seizures. Vascular remodeling was assessed in mesenteric arteries (MA) using pressurized myography. Reactive oxygen species (ROS) production by dihydroethidium staining, vascular cell adhesion molecule (VCAM)-1 expression and CD3+ T cell infiltration by immunofluorescence were determined in aorta or perivascular adipose tissue (PVAT). BP was measured by telemetry after 6 weeks of HSD.

**Results:** Systolic BP tended to be higher in SS2BNb compared to SS (185±8 vs 168±5 mmHg). The incidence of seizures was 3.9-fold higher in SS2BNb compared to SS (12/36 vs 3/35 rats (P<0.05). MA media/lumen was 1.3-fold higher in SS2BNα compared to SS (10.6±0.9 vs 7.9±0.4%, P<0.01). PVAT ROS production was 1.8-fold higher in SS2BNα compared to SS (5.6±0.8 vs 3.2±0.1 relative fluorescence units [RFU]/µm², P<0.01) and tended to be lower in SS2BNb (2.1±0.4 vs 3.2±0.1 RFU/µm²). Aortic VCAM-1 was increased 2.1-fold in SS2BNα compared to SS (2.6±0.5 vs 1.2±0.3 RFU/µm², P<0.05). Aortic PVAT CD3+ T cell infiltration was 55% lower in SS2BNb compared to SS (17±4 vs 38±4 cells/mm², P<0.05).

**Conclusions:** Unexpectedly, SS2BNα rats present increased vascular injury under HSD. The absence of vascular inflammation and remodeling in SS2BNb rats despite slightly higher BP seems maladaptive and may explain the increased incidence of stroke.

TRPM7 is a cationic ion channel and with a serine/threonine kinase important for cellular Mg²⁺ homeostasis. We recently showed that TRPM7-kinase plays a role in aldosterone-mediated vascular effects and inflammation. Here we explored the role of TRPM7-kinase in cardiac fibrosis and vascular function in aldosterone-induced hypertension in mice. Wild-type (WT) or heterozygote TRPM7-kinase domain (TRPM7+/-) were treated with infused aldosterone (600 µg/Kg/day) and NaCl 1% in drinking water (aldo/salt) for 4 weeks. Blood pressure (BP) was evaluated by tail-cuff. Vessel function was investigated in mesenteric arteries by wire and pressure myography. Protein expression was assessed in cardiac tissue by western-blot and histology. Aldo/salt increased BP in TRPM7+/- and WT to similar levels (137mmHg vs control 118mmHg). Mesenteric arteries from untreated TRPM7+/- mice were more sensitive to relaxation induced by acetylcholine (LogEC50: 7.6±0.1 vs 7.1±0.2, TRPM7+/- and WT, respectively), effects that
were reduced by Aldo/salt treatment (LogEC50: 7.2±0.1). Phenylephrine-contraction and sodium nitroprusside-relaxation curves were similar among groups. Pressure myography showed that in WT, Aldo/salt increase the diameter (26%) and cross-sectional area (40%), resulting in hypertrophic outward remodelling, whereas in TRPM7+-/-, the treatment decreased the diameter (16%) and increase the wall/lumen ration (82%), resulting in eutrophic inward remodelling. Hearts from TRPM7+-/+ presented decreased expression of annexin-1, which is a target protein of TRPM7-kinase, that was further decreased by Aldo-salt. Hearts from untreated TRPM7+-/- mice had increased fibrotic markers: plasma galectin-3 (2.5ng/mL vs WT (1.4ng/mL) and protein expression for fibronectin (2.4-fold) and TGFβ (2-fold), and the aging marker p-P66Sch (47%) which were similar to WT-Aldo/salt. Aldo/salt induced higher collagen expression in TRPM7+-/- than in WT animals (15%), as observed by picrosirius red staining. Our findings provide some insights into aldosterone signalling through TRPM7-kinase and suggest that this chanzyme may have protective actions, which when downregulated, promotes vascular remodelling and cardiac fibrosis in aldosterone-induced hypertension.


Funding: No
Funding Component: P229

Chymase-mediated Igf-1 Degradation Promotes Delayed Cell Death in Post-ischemic Hearts

Lin Tan, Thor Tejada, Rebecca Torres, John Calvert, Emory Univ, Atlanta, GA; Gunnar Pejler, Uppsala Univ, Uppsala, Sweden; Magnus Abrink, Swedish Univ of Agricultural Sciences, Uppsala, Sweden; David Lefer, Ahsan Husain, Nawazish Naqvi, Emory Univ, Atlanta, GA

Heart disease is a leading cause of death in adults. Here we show that a few days after coronary artery ligation and reperfusion, the ischemia-injured heart elaborates the cardioprotective polypeptide, insulin-like growth factor-1 (IGF-1), which activates IGF-1 receptor prosurvival signaling and improves cardiac left ventricular systolic function. However, this is antagonized by the chymase, mouse mast cell protease-4 (MMCP-4), which degrades IGF-1 (Fig. 1). We found that MMCP-4 deficiency, resulted in sustained IGF-1 levels and IGF-1 receptor prosurvival signaling post-I/R. MMCP-4 deficiency markedly reduced late, but not early, infarct size (~50% reduction: n=5-7, p value= 0.001) by suppressing IGF-1 degradation and, consequently, improving cardiac function (EF: 26% greater, n=21, p value= 0.001) and adverse structural remodeling (Fig. 2). Our findings represent the first demonstration of tissue IGF-1 regulation through proteolytic degradation and suggest that chymase inhibition may be a viable therapeutic approach to enhance late cardioprotection in post-ischemic heart disease.
Background: Dicrotic Notch (DN) is known to dampen with age, with increasing arterial stiffness probably due to arterial calcification. Since arterial calcification has recently been shown to predominantly involve descending thoracic aorta, we hypothesized that calcification in different segments of thoracic aorta will have a different impact on DN.

Methods: A sample of 44 patients with invasive thoracic aortic pressure tracings during cardiac catheterization was selected for this study. Non-contrast CT scans were evaluated for presence of calcification in aortic segments (ascending aorta (AA), aortic arch (arch) and descending aorta (DA)) and then quantified. DN was categorized based on aortic pressure tracings into 4 grades. Grade 1 represented normal DN; grades 2, 3 and 4 represented progressively diminishing DN, where grade 4 represented absent DN. Compliance was calculated as a change in stroke volume over aortic pulse pressure with both measurements obtained from echocardiography reports done within one year of catheterization. Results: The mean age of the sample population was 64.6 ± 10.5 years. Out of the 44 patients, 14 (32%) had a calcified AA, 25 (56%) had a calcified DA and 28 (63%) had a calcified arch. Furthermore, 14 (32%) patients had only one segment.

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P230

The Influence of Calcification of Ascending Aorta on Dicrotic Notch of Thoracic Aorta

Aahad Khan, Scott Ray, Syed Haris Pir, Mustafa Noor Muhammad, Mirza Mujadil Ahmad, Sharmeen Hussaini, Mirza Nubair Ahmad, Imaad Razzaque, Muhammad Nabeel Syed, Rafath Ullah, Khawaja Afzal Ammar, Aurora St. Luke’s Medical Ctr, Milwaukee, WI

Figure 1: Identification of MM014:4 as the major ISI-1-degrading protease in post-IR hearts. (A) Fractions 72 to 85 WT heart was incubated with recombinant mouse KIF-1 (nis-IR-I) and then subjected to SDS-PAGE and immunoblotting. S1 and S2 are high-speed supernatant fraction and membrane pellet, respectively. S2 and R0 are 1% Triton X-100 supernatant fraction and pellet, respectively, derived from WT. 24 h post-IR fraction 1 and 3 are 1% Brij supernatant fraction and pellet, respectively, derived from WT. Soluble recombinant ISI-1-degrading activity is evident in the highspeed supernatant fraction of WT hearts. (B, D) Immuno blot showing that ISI-1-degrading activity in 3T3 is ablated with anti-(human), anti-MyD88, or total protein extract (Silver-stained, Coomassie blue). (C) Expression analysis of murine MyD88 by real-time PCR showed that MyD88 levels were increased in WT heart (control) and decreased in 24 h post-IR hearts. (D) Isoproterenol treatment for 24 h post-IR hearts was used to determine the extent of ISI-1-degrading activity in WT and MyD88−/− hearts. (E) Isoproterenol treatment for 24 h post-IR hearts was used to determine the extent of ISI-1-degrading activity in WT and MyD88−/− hearts.
calcified, whereas 10 (23%) had two and 11 (25%) had all three segments calcified. Abnormal DN was present in 16 (36%) patients. The odds of having an abnormal DN in the presence of calcified AA were more than 3 times (OR: 3.67; p=0.05). Compliance was higher in those with a normal DN versus those with an abnormal DN (1.64 ml/mmHg vs. 1.21 ml/mmHg) (p = 0.09). There was no significant association between calcification in the DA or arch of aorta. **Conclusion:** There was no association between dicrotic notch and presence of calcification in the arch of the aorta and descending aorta.


**Funding:** No

**Funding Component:** P231

**Inhibition of MiR-762 Prevents and Reverses Ang II Induced Aortic Stiffening**

Kim Ramil C Montaniel, Jing Wu, Vanderbilt University, Nashville, TN; Matthew R Bersi, Yale Univ, New Haven, CT; Liang Xiao, Hana A Itani, Kasey C Vickers, Vanderbilt University, Nashville, TN; Jay D Humphrey, Yale Univ, New Haven, CT; David G Harrison, Vanderbilt University, Nashville, TN

We and others have shown that hypertension (HTN) is linked with striking fibrosis in the aortic adventitia. This leads to aortic stiffening, leading to organ damage. Through a screen of microRNAs (miRNAs) in the aorta, we found that miR-762 is the most upregulated miRNA in Ang II hypertensive mice. qRT-PCR confirmed that miR-762 is upregulated 6.35±1.22 (p=0.03) fold in Ang II-infused mice compared to controls. To study the role of miR-762 in HTN, we administered a locked nucleic acid inhibitor of miR-762. MiR-762 inhibition normalized stress-strain relationships and aortic systolic energy storage (ASE) (Table). Moreover, miR-762 inhibition in the last 2 weeks of Ang II infusion reversed aortic stiffness in mice treated with 4 wk of Ang II (ASE, 4 wk Ang II [51±5.18 kPa] vs 4wk Ang II + LNA-762 [last 2 wk] [20±1.76 kPa], p<0.0001). Further studies showed that miR-762 inhibition reduced mRNA for several collagens and fibronectin and upregulated collagenases MMP1a, 8 and 13 (Table). Lastly, we found that miR-762 inhibition during Ang II infusion led to a 9.11±1.92 (p=0.007) fold increase in Sprouty1 mRNA, suggesting that miR-762 targets Sprouty1 mRNA. Sprouty1 inhibits the activation of p38-MAPK which is critical in the process of aortic stiffening. Hence, miR-762 modulates aortic stiffening and fibrosis through a Sprouty1-p38-MAPK mechanism. Thus, miR-762 has a major role in modulating aortic stiffening and its inhibition dramatically inhibits pathological fibrosis, enhances matrix degradation, prevents and reverses aortic stiffness. miR-762 inhibition might represent a new approach to prevent aortic stiffening and its consequent end-organ damage.

**Table: The effect of miR-762 inhibition on aortic stiffness and matrix gene expression.** All values are presented as mean±SE. *Note: All fold change values were normalized to the sham group.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sham</th>
<th>Ang II</th>
<th>Ang II + LNA-762</th>
<th>P value (Dunnett ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced Col</td>
<td>0.57±0.05</td>
<td>2.3±0.15</td>
<td>79.97±7.113</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>Syntelic energy</td>
<td>86±2.49</td>
<td>17±1.33</td>
<td>61±4.73</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Collagen 1a</td>
<td>1.00±0.05</td>
<td>8.5±0.25</td>
<td>3.0±0.04</td>
<td>0.0140</td>
</tr>
<tr>
<td>Collagen 3a</td>
<td>1.00±0.0</td>
<td>8.9±0.27</td>
<td>0.00002</td>
<td>0.0001</td>
</tr>
<tr>
<td>Collagen 5a</td>
<td>1.00±0.04</td>
<td>3.3±0.61</td>
<td>0.5±0.07</td>
<td>0.0033</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>1.00±0.36</td>
<td>2.1±0.18</td>
<td>0.00004</td>
<td>0.0005</td>
</tr>
<tr>
<td>MMP1α</td>
<td>1.00±0.21</td>
<td>1.8±1.17</td>
<td>0.109±0.06</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>MMP13</td>
<td>1.00±0.34</td>
<td>5.2±2.83</td>
<td>120.7±29.09</td>
<td>&gt;0.0001</td>
</tr>
</tbody>
</table>

Psoriasis is Associated with Increased Arterial Stiffness: a Systematic Review and Meta-analysis

Sikarin Upala, Anawin Sanguankeo, Bassett Medical Ctr, Cooperstown, NY

Background Studies have shown that patients with psoriasis have higher risk of CVD, independent of traditional CVD risk factors. However, pathophysiology of the development of CVD in psoriasis is not well known. Arterial stiffness has been recognized as an independent predictor of cardiovascular risk. It is controversial whether psoriasis and arterial stiffness is associated. In this systematic review and meta-analysis, we sought to assess the hypothesis that patients with psoriasis have increased arterial stiffness compared with controls. Methods Systematic literature search was performed using MEDLINE and EMBASE databases from inception to May 2016. We included original research publications that contained data on arterial stiffness and psoriasis. Aortic pulse wave velocity (aPWV) is the non-invasive marker for assessment of arterial stiffness. We compared aPWV between patients with psoriasis and controls and estimated the pooled mean difference (MD) and 95% confidence interval (CI) of aPWV using a random-effects model meta-analysis. Results Data from five observational studies involving 438 participants (233 with psoriasis) were extracted and included in the meta-analysis. Pooled MD of aPWV was 1.17 m/sec higher in patients with psoriasis compared with controls (95% CI: 0.78-1.55, P-value<0.01, I² = 69%). There is no change in the direction or statistical significance of MD of aPWV after removing each study at a time in the sensitivity analysis. Conclusion Psoriasis is associated with increased arterial stiffness. Assessment of arterial stiffness parameters may be important for early detection of cardiovascular deterioration in psoriasis patients.

Diabetic Cardiomyopathy is Reversed by Increased Mitochondrial Bioenergetics Due to PGC-1α Activation by EET Treatment of Obese Mice

Jian Cao, First Dept of Geriatric Cardiology, Chinese PLA General Hosp, Beijing, China; John A. McClung, Dept of Cardiology, New York Medical Coll, Valhalla, NY; Shailendra P. Singh, Lars Bellner, Dept of Pharmacology, New York Medical Coll, Valhalla, NY; Maayan Waldman, Leviev Heart Ctr Sheba Medical Ctr, Tel Hashomer and Sackler Sch of Med, Tel-Aviv Univ, Petach-Tikva, Israel; Joseph Schragenheim, Dept of Pharmacology, New York Medical Coll, Valhalla, NY; Michael Arad, Cardiac Res Lab, Felsenstein Medical Res Inst, Tel-Aviv Univ, Petach-Tikva, Israel; Joseph I. Shapiro, Dept of Med, Joan C.
Introduction: Obesity and diabetes are associated with progressive cardiac fibrosis that, sequentially, results in diastolic dysfunction, reduced contractility, and ultimately heart failure. Contributing factors include hyperglycemia, insulin resistance, mitochondrial dysfunction, and a reduction in AMPK signaling. PGC-1α activates mitochondrial biogenesis and oxidative phosphorylation and is decreased in patients with diabetes mellitus (DM). We hypothesize that an epoxyeicosatrienoic acids (EETs) agonist (EET-A) will increase PGC-1α levels in a db mouse model of DM attenuate cardiomyopathy, and prevent heart failure.

Methods: Db mice (4-wks), were allowed to acclimatize for 16-wks and were then divided into 3 treatment groups for an additional 16 wks: A) control, B) EET-A 1.5mg/100g BW 2 weeks and C) EET-A-Ln-PGC-1α shRNA. Ln-PGC-1α shRNA suppressed PGC-1α protein in heart tissue by 40-50%. Oxygen consumption (VO2), and blood glucose was determined. Heart tissues were harvested to measure PGC-1α, HO-1, pAMPK, PGC-1α, echocardiographic fractional shortening, mitochondrial oxidative phosphorylation (OXPHOS) and mitofusion protein markers.

Results: All mice developed heart failure by the end of 16 weeks and were characterized by a decrease in myocardial contractility, an increase in insulin resistance and blood pressure, decreased VO2, the appearance of mitochondria dysfunction and a decrease in AMPK and downstream PGC-1α signaling. Mice treated with EET-A demonstrated an increase in PGC-1α levels, improved mitochondrial function and oxidative phosphorylation (p<0.01 vs control), increased NO bioavailability (p<0.05 vs control), and normalization of glucose metabolism, insulin levels, VO2 and LV systolic function (p<0.05 vs control). All of these findings were suppressed by PGC-1α inhibition which was accompanied by the onset of even more severe LV dysfunction than in the control group. Conclusion: Increased EET levels result in activation of PGC-1α-HO-1 which reverses diabetes induced insulin resistance, mitochondrial dysfunction, and cardiomyopathy. EET may have potential as a powerful agent for therapeutic application in the treatment of diabetic cardiomyopathy.
mice hearts and 50% in failing human hearts compared to their controls respectively. Mice overexpressing CTMP specifically in the heart were resistant to AB-induced cardiac hypertrophy, whereas cardiac-specific conditional CTMP-knockout mice exhibited an aggravated phenotype induced by pressure overload. Additionally, gain-or-loss of function experiments mediated by adenovirus demonstrated that CTMP also prevented an angiotensin II–induced hypertrophic response in isolated cardiomyocytes in vitro. Mechanistically, we discovered that AKT signaling was significantly activated in AB-treated WT hearts, which was blocked by cardiac overexpression of CTMP, whereas being enhanced by loss of CTMP in response to chronic pressure overload and agonist stimulation. Moreover, rescue-experiments revealed that inhibition of AKT activation through LY294002 ameliorated the cardiac abnormalities in CTMP-knockout mice after AB. Taken together, our present study provides both in vitro and in vivo evidences that CTMP functions as a novel negative regulator factor of pathological cardiac hypertrophy. The underlying mechanisms responsible for CTMP-elicited effects are dependent on the inhibition of AKT signaling. The above-mentioned findings also expand our knowledge of the mechanisms of cardiac hypertrophy and provide potential therapeutic targets for pathological cardiac hypertrophy and heart failure.

H. Li: None. K. Deng: None. X. Zhang: None.

Funding: No

Funding Component: P235

Balancing Truss Expression Paves a Way for Cardiac Hypertrophy Therapy

Hongliang Li, Xiao-Jing Zhang, Ke-Qiong Deng, Wuhan Univ, Wuhan, China

Pathological cardiac hypertrophy, which is always accompanied by cardiac fibrosis and the resultant cardiac dysfunction, leads to heart failure and even sudden death. The TNF-receptor ubiquitous signaling and scaffolding protein (TRUSS) that is enriched in the heart has been identified as a negative regulator of cancer. However, the role of TRUSS in cardiac remodeling is unknown. Here, we aimed to investigate the potential participation of TRUSS in cardiac hypertrophy and the molecular events by which TRUSS regulates this pathological condition. The pathological cardiac hypertrophy model was established by pressure overload in vivo and Ang II stimulation in vitro. We observed that the expression level of TRUSS was dramatically increased in the heart and in primary cardiomyocytes upon pro-hypertrophic stimuli. To illustrate the functional role of TRUSS in cardiac remodeling, the cardiac specific knockout (KO) or transgenic (TG) mice were employed. After aortic binding (AB) for 4 weeks, TRUSS deficiency conferred significant resistance to pressure overload via significantly inhibiting cardiomyocytes enlargement and fibrosis formation by about 37% and 46%, respectively, whereas dramatically exacerbated hypertrophy, fibrosis, and cardiac dysfunction were shown in TRUSS-TG mice compared to their littermate controls. Mechanistically, TRUSS can directly bind to JNK, a well-known pro-hypertrophic factor, and activate its downstream pathway. Further investigations indicated that the aggravated effect of TRUSS on cardiac hypertrophy can be almost completely reversed by a specific JNK inhibitor, SP600125, indicating a JNK-dependent manner of TRUSS-regulated cardiac hypertrophy. The directly exacerbated function of TRUSS in cardiomyocytes and the JNK-dependent mechanisms were further validated in primary cardiomyocytes that treated with Ang II after infection with AdshTRUSS or AdTRUSS. Notably,
the increased protein and mRNA expression of TRUSS was also observed in heart samples from patients with hypertrophic cardiac myopathy. In conclusion, TRUSS functions as a positive regulator of pathological cardiac hypertrophy, suggesting a promising therapeutic approach for the hypertrophy related heart diseases by balancing TRUSS expression.

H. Li: None. X. Zhang: None. K. Deng: None.
Funding: No
Funding Component: P236

Tripartite Motif 8 Contributes to Pathological Cardiac Hypertrophy Through Enhancing TAK1-dependent Signaling Pathways

Hongliang Li, Yan-Xiao Ji, Peng Zhang, Wuhan Univ, Wuhan, China

Tripartite motif (TRIM) 8 functions as an E3 ligase, interacting with and ubiquitinating diverse substrates, and is implicated in various pathological processes. However, the biological function of TRIM8 in the heart remains largely uncharacterized. This study aims to explore the role of TRIM8 in the development of cardiac hypertrophy and heart failure (HF). Mice and isolated neonatal rat cardiomyocytes (NRCMs) overexpressing or lacking TRIM8 were examined in several experiment. The effect of aortic banding (AB)-induced cardiac hypertrophy were analyzed by echocardiographic, pathological and molecular analyses. Our results indicated that the TRIM8 overexpression in hearts exacerbated the pathological cardiac hypertrophy triggered by AB, promoting cardiomyocytes enlargement and fibrosis formation by about 41% and 52%. In contrast, the development of pathological cardiac hypertrophy was profoundly blocked in TRIM8-deficient hearts. Mechanistically, the present study suggests that TRIM8 may elicit cardio-detrimental effects by promoting the activation of TAK1-p38/JNK signaling pathways. Similar results were observed in cultured NRCMs treated with angiotensin II. In addition, the rescue experiments using the TAK1-specific inhibitor 5z-7-ox confirmed the requirement of TAK1 activation in pressure overload-mediated pathological cardiac hypertrophy in TRIM8-overexpressing hearts. Furthermore, a physical interaction between TRIM8 and TAK1 was identified by co-immunoprecipitation experiments. Our study demonstrated that TRIM8 plays a deleterious role in pressure overload-induced cardiac hypertrophy by accelerating the activation of TAK1-dependent signaling pathways.

H. Li: None. Y. Ji: None. P. Zhang: None.
Funding: No
Funding Component: P237

Is Ischemia/Reperfusion an Efficient Method for Producing Heart Failure in Rats?

Ana Carolina M Omoto, Fábio N Gava, Mauro de Oliveira, Carlos A Silva, Rubens Fazan Jr., Helio C Salgado, Ribeirão Preto Medical Sch, Ribeirão Preto, Brazil

Myocardium infarction (MI) elicited by coronary artery ligation (CAL) is commonly used to induce chronic heart failure (HF) in rats. However, CAL shows high mortality rates. Given that ischemia-reperfusion (IR) may cause the development of HF, this approach may be useful for obtaining a model of HF with low mortality rates. Therefore, it was compared the model of CAL vs. IR in rats, evaluating the mortality and cardiac morphological and functional aspects. The IR consisted of 30 minutes of cardiac ischemia. Wistar rats were assigned into three groups: CAL: n=18; IR: n=7; SHAM (fictitious IR): n=7. After four weeks of CAL, the subjects were evaluated by echocardiography and ventriculography as well.
The statistical analysis consisted of ANOVA combined with Tukey's posthoc test (p<0.05). There were no deaths in the IR and SHAM groups, whereas in the CAL group the mortality rate was 33.33% (6 out of 18). In the CAL group echocardiography showed increased left ventricular (LV) cavity during systole (8.3 ± 1mm) and diastole (10.5 ± 1mm); decreased LV free wall during systole (1.4 ± 0.5 mm); increased left atrium/aorta (2.3 ± 0.4) ratio. These changes were not significant in IR (4.8 ± 0.5mm, 7.6 ± 0.6mm, 2.6 ± 0.3 mm, 1.6 ± 0.2) and SHAM (4.6 ± 0.6 mm, 7.7 ± 0.8mm, 2.8 ± 0.4mm, 1.5 ± 0.2) groups. There was also the reduction in the ejection fraction in the CAL group (41 ± 12 %) when compared with IR (65 ± 9%) and SHAM (69 ± 7%) groups. The tissue Doppler analysis from the lateral mitral annulus showed reduction in E´ in CAL (-29 ± 8 mm/s) and IR (-31± 9 mm/s) groups when compared with the SHAM (-48 ± 11 mm/s) group. The ventriculography in the CAL group showed smaller maximum dP/dt (6519 ± 1062) and greater end-diastolic pressure (33 ± 8 mmHg) when compared with IR (8716 ± 756 mmHg/s; 9 ± 9 mmHg) and SHAM (7989 ± 1230 mmHg/s; 9 ± 7 mmHg) groups. The CAL group presented transmural infarct size of 40% of the left ventricular wall, measured under histopathological examination. In conclusion, IR for 30 minutes caused only small changes in LV diastolic function, assessed by tissue Doppler; however, the IR was not effective for promoting HF, as observed with CAL. Thus, it is possible that prolonged IR is necessary for promoting significant HF in rats.


Funding: No
Funding Component: P238

Effect of Zamicastat in Chronic Treatment on Right Ventricle Pressure Overload in the Rat Monocrotaline Lung Injury Model of Pulmonary Hypertension

Bruno Igreja, Nuno Pires, Paul Moser, Patrício Soares-da-Silva, BIAL - Portela & Companhia, SA, Trofa, Portugal

Pulmonary arterial hypertension (PAH) is defined, foremost, as a plexogenic arteriopathy with subtotal luminal obliteration that increases pulmonary vascular resistance and imposes a hemodynamic stress on the right ventricle (RV) leading to RV hypertrophy and failure that contributes to premature death. There is experimental and clinical evidence that supports a relation between PAH and the sympathetic nervous system (SNS), indicating that PAH can be mediated, at least partly, by SNS hyperactivation. A strategy for the modulation of sympathetic nerve function is to reduce the biosynthesis of norepinephrine (NE) by inhibiting dopamine β-hydroxylase (DβH), the enzyme that catalyzes the conversion of dopamine (DA) to NE in sympathetic nerves. Here, we evaluated the effect of 18-day oral treatment with the DβH inhibitor zamicastat on RV pressure overload in the monocrotaline (MCT)-induced pulmonary hypertension (PH) model in the rat. MCT increased RV systolic pressure (54.7±2.9 vs 32.3±1.0 mmHg, p<0.0001), mean RV pressure (23.9±1.7 vs 16.6±1.1 mmHg, p<0.001), and decreased heart rate (240.0±5.9 vs 285.8±7.6 beats/min, p<0.0001) in vehicle-treated rats, as compared to pre-MCT values. Zamicastat treatment prevented the MCT-induced increase in mean RV pressure (change from baseline: +1.9±1.5 vs +7.4±1.8 mmHg, p<0.05). Likewise, the HR decrease was significantly attenuated in the zamicastat group as compared to the vehicle group (change from baseline: -14.3±9.7 vs -45.8±8.1 beats/min, p<0.05). Chronic
zamicastat treatment decreased NE levels (480.5±43.9 vs 712.1±46.5 ng/mg protein, p<0.005) and increased DA levels (533.0±49.7 vs 12.3±2.3 ng/mg protein, p<0.0001) in adrenal gland homogenates of MCT-treated rats, as compared to vehicle group demonstrating robust inhibition of DβH. In conclusion, the DβH inhibitor zamicastat reverses heart rate and RV pressure changes, two hallmarks of PAH, in the rat MCT lung injury model.

**B. Igreja:** A. Employment; Modest; BIAL - Portela & Cª S.A.  
**N. Pires:** A. Employment; Modest; BIAL - Portela & Cª S.A.  
**P. Moser:** A. Employment; Modest; BIAL - Portela & Cª S.A.  
**P. Soares-da-Silva:** A. Employment; Modest; BIAL - Portela & Cª S.A.

**Funding:** No  
**Funding Component:** P239

**Papaverine-induced Ventricular Fibrillation Developing During Coronary Flow Reserve Studies**

**Yoshitaka Okabe**, Kannichi Otowa, Yasuhiro Mitamura, Manabu Kikyoutani, Tsuruga City Hosp, Tsuruga, Japan

**Background:** Estimation of the fractional flow reserve (FFR) is considered to be an established method by which to assess stable coronary artery stenosis. Induction of maximal coronary hyperemia is important during FFR. Papaverine is often used to achieve maximal hyperemia. However, this drug has been reported to increase the risk of ventricular arrhythmias. The purpose of the present study was to discover predictors of papaverine-induced ventricular fibrillation (VF) developing during FFR.

**Methods:** A total of 187 clinically stable patients were included in the study. FFR was performed to evaluate lesions for which percutaneous coronary intervention (PCI) was to be considered after coronary angiography. FFRs were determined after intracoronary papaverine administration (12 mg into the left and 8 mg into the right coronary arteries). We compared patients in whom VF did and did not develop in terms of clinical and ECG characteristics. Results: We performed FFR on 214 lesions (112 in the left anterior descending arteries, 38 in the left circumflex arteries, and 64 in the right coronary arteries) of 187 patients. The average patient age was 72.5 ± 10 years. We found that the QTc interval was prolonged in all patients after papaverine administration (average post-administration QTc interval = 569 ± 89 ms; average ΔQTc interval = 144 ± 80 ms). VF developed in three patients with significantly prolonged QT intervals (average post-administration QTc interval = 639 ± 19 ms, average ΔQTc interval = 220 ± 64 ms, p < 0.02) and all of them occurred after administration of papaverine into the left coronary artery. Three-vessel disease was significantly predictive of VF (p < 0.003). In the three-vessel group, the complications of low left ventricular function (ejection fraction<50%), hypokalemia (serum K <3.5 mEq/L), and bradycardia (<50 beats/min), were significantly associated with VF (p < 0.045). Conclusions: Three-vessel disease is a predictor of the development of VF during FFR performed with the aid of papaverine, especially if accompanied by one or more of low left ventricular function, hypokalemia, or bradycardia.

**Y. Okabe:** None. **K. Otowa:** None. **Y. Mitamura:** None. **M. Kikyoutani:** None.

**Funding:** No  
**Funding Component:** P240

**Acetylcholine Stimulation with Pyridostigmine Prevents Cardiac Arrhythmias in Conscious Myocardial Infarcted Rats and Upregulate Connexin 43, Hipoxic Inducible Factor-1α and**
Vascular Endothelial Grow Factor in Cardiomyocytes Culture

Fernanda M Santos-Almeida, César A Meschiari, Univ of Sao Paulo, Ribeirao Preto, Brazil; Tânia Martins-Marques, Univ of Coimbra, Coimbra, Portugal; Maria L Cury-Pavão, Helio C Salgado, Univ of Sao Paulo, Ribeirao Preto, Brazil; Henrique Girão, Univ of Coimbra, Coimbra, Portugal; Rubens Fazan, Univ of Sao Paulo, Ribeirao Preto, Brazil

Objective: To examine the impact on hemodynamics and electrocardiogram (ECG) of treating acutely infarcted rats with the acetylcholinesterase inhibitor pyridostigmine (PYR). We also examined the effect of PYR on connexin 43 (Cx43), an important protein for the ventricular cell to cell communication; on hypoxic inducible factor (HIF-1α), a transcriptional factor related to cell survival pathways under hypoxia, and on vascular endothelial grow factor (VEGF) in cultured cardiomyocytes subjected to ischemia-mimetic conditions.

Methods and Results: Wistar rats, previously implanted with ECG electrodes and catheters into femoral artery and vein were subjected, under inhaled anesthesia, to coronary artery ligation to elicit MI, and after 20 min they received PYR (0.12 mg/kg iv, N=8) or saline (N=7). After 3h, the animals had their arterial pressure (AP) and ECG recorded for 60 min. Additionally, H9c2 cells were incubated with PYR (0.5 mM) during 1h before being subjected to the ischemia-mimetic solution during 1h. Mean AP was found similar between groups (94±5 vs. 90±3 mmHg) while heart rate (HR) was lower in rats treated with PYR (390±11 vs. 441±12 bpm). The number of premature ectopic beats was markedly lower in rats that received PYR, as compared to saline treated counterparts (median: 7 vs. 27, 25th percentile: 1.75 vs. 8.5 and 75th percentile: 17 vs. 166). In cultured cells incubated with PYR Cx43 was found higher (0.72±0.16 vs. 0.49±0.16), as well as HIF-1α (3.88±0.41 vs. 2.23±0.5), and mRNA for VEGF (qualitative analysis).

Conclusion: Treatment with PYR showed positive effects, reducing the tachycardia and arrhythmias commonly seen after acute MI. Additionally, PYR affected proteins related to cell communication, cell surviving and vascular grow in cultured cardiomyocytes subjected to ischemia-mimetic conditions. The in vitro effects of PYR might be linked to its beneficial effects observed in infarcted rats. In another view, the results open a very new field of cholinergic role on cardiomyocytes to be further investigated.


Funding: No

Funding Component: P241

Irisin Protects Mitochondria Function During Pulmonary Ischemia Reperfusion Injury

Chunyu Zeng, Ken Chen, Daping Hosp, Chongqing, China

Background: Ischemia and reperfusion (I/R) induced lung injury is one of the most important and common causes of early and high morbidity and mortality. The mitochondrial damage of alveolar epithelium cells leads to
further damage in lung I/R by augmented ROS activity and inflammation. Previous study show a humoral myokine, irisin, has been found stimulate mitochondrial biogenesis. We hypothesize irisin might protect lung from I/R injury. Methods The lung I/R injury model was performed on C57BL/6J mice. The pulmonary protection of exogenous irisin was indicated by lung edema and function measurement, and the survival of mice. The localization of irisin was measured by immunohistochemistry and immunofluorescence staining. And the protective effect of irisin to mitochondrial was demonstrated by ATP production, ROS production, mitochondrial-dependent apoptosis measurement, and so on. Results There was no endogenous irisin expression in the alveolar wall in normal mice. However, it is interesting to find that, irisin was in the alveoli after lung I/R injury. The increased irisin in lung might be from plasma, because in the mean time, the plasma irisin levels were decreased. Exogenous intravenous injection of irisin protect the lung from I/R injury. The protective effect might be via the protection on the mitochondria in lung, exogenous irisin was found to localize in the mitochondria, the impaired mitochondrial function in I/R mice was reversed after irisin treatment. Moreover, there was colocalization between the irisin and UCP2, exogenous irisin decreased the I/R-induced UCP2 degradation. The role of UCP2 on the protection of mitochondria is further confirmed in the in vivo experiment, inhibition of UCP2 or knockout of UCP2 would make the protection of irisin on lung function and mitochondrial function lost. Conclusions Administration with exogenous irisin would alleviate the I/R damage, reduced the inflammatory and superoxide factors, ameliorated the mitochondrial dysfunction, via the binding of irisin and UCP2 in lung.

C. Zeng: None. K. Chen: None.

Funding: No

Funding Component: P242

Microvascular Changes in Brain During Salt-sensitive Hypertension: Molecular Mechanisms and Implications for Small Vessel Disease

Travice Michael De Silva, Justin Grobe, Frank Faraci, Univ of Iowa Coll of Med, Iowa City, IA

Hypertension is a major risk factor for small vessel disease (SVD), a leading contributor to stroke and dementias. Mechanisms that underlie SVD in brain are poorly defined, with no specific therapy at present. Because parenchymal and pial arterioles are targets of the SVD process, we examined microvascular changes in a model using deoxycorticosterone-salt (DOCA) to activate the brain renin-angiotensin system (RAS), with resulting salt-sensitive (sodium- and fluid-dependent) hypertension. Male C57Bl/6 mice were treated with DOCA and given the choice of drinking H2O or H2O with 0.9% NaCl for 3 wks. Along with a modest elevation in mean arterial pressure (79±2 vs 95±3 mmHg, P<0.05), DOCA impaired endothelium-dependent dilation of both isolated parenchymal (baseline diameter of 15±1 µm) and pial arterioles (37±1 µm) in a pathway specific manner. Endothelium-dependent hyperpolarization was intact while eNOS-mediated vasodilation was markedly impaired along with reductions in phosphorylation in AKT (an upstream activator of eNOS). Local inhibition of angiotensin II type 1 (AT1-R) or mineralocorticoid receptors (MR) or Rho kinase (including ROCK2), restored endothelial function in DOCA-treated mice. Inner diameter of maximally dilated parenchymal arterioles was reduced approximately 20% by DOCA (P<0.05 vs sham). DOCA increased mRNA expression of RAS components (eg, AGT, ACE) in both brain and cerebral vessels. In NZ44 reporter mice that express GFP driven by the AT1A-R promoter,
DOCA increased cerebrovascular GFP protein expression about 3-fold (P<0.05). Thus, DOCA activates both the brain and the cerebrovascular RAS, impairs select pathways affecting parenchymal and pial arteriolar function, while producing inward microvascular remodeling. AT1R, MR and ROCK2 are key contributors to cerebral microvascular dysfunction in this clinically relevant model of SVD.

T.M. De Silva: None. J. Grobe: None. F. Faraci: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, HL- 62984, HL-113863, NS-096465.

Funding: No

Funding Component: P243

A New Type 2 Diabetic Rat Model That is Associated with Cognitive Impairment in Aging

Wenshan Lv, Univ of Mississippi Medical Ctr, Jackson, MS; Hongwei Yu, Medical Coll of Wisconsin, Milwaukee, WI; Longyang Li, Christine Taylor, Ezekiel Gonzalez-Fernandez, Jerrrell Sims, Matthew R Elliott, Univ of Mississippi Medical Ctr, Jackson, MS; Yangang Wang, Affiliated Hosp of Qingdao Univ, Qingdao, China; Jan M Williams, Richard J Roman, Fan Fan, Univ of Mississippi Medical Ctr, Jackson, MS

Alzheimer’s disease (AD) is an incurable neurodegenerative disease and the most common form of dementia, and AD and type II diabetes (DM II) are two of the most common diseases of aging. Numerous studies demonstrate DM II with increased risk for dementia, however, the mechanisms linking DM II and AD have not been fully elucidated. There is increasing evidence suggesting that cerebral vascular dysfunction plays an important role in the development of AD. T2DN rat is a DM II rat model that exhibits diabetic nephropathy. The present study examines whether aged T2DN rat is associated with cognitive impairment, and whether autoregulation of cerebral blood flow (CBF) is impaired that contributes to AD. The levels of glucose (422 ± 32 vs. 94 ± 3 mg/dL) and glycated hemoglobin (HbA1c, 11.5 ± 0.2 vs. 4.3 ± 0.1%) were higher in 12-18 months old T2DN than in age matched SD control rats. CBF rose by 137 ± 15% and 36 ± 5%, respectively, in T2DN and SD rats when MAP was increased from 100 to 180 mmHg. Aged T2DN rats exhibited BBB leakage and “AD” like cerebral vascular remodeling. The expression of Amyloid β 42 (Aβ42), p-tau (S416), GFAP and IL-1 beta were significantly higher in the brains of T2DN vs. SD rats. T2DN rats also exhibited learning and memory dysfunction as the short term (2-hour; T2DN 96 ± 12 vs. SD 13 ± 3 seconds) and long term (24-hour; T2DN 105 ± 15 vs. SD 8 ± 2 seconds) latency of escape were longer in an eight-arm water maze test, and spent less time in the target arm 48 hours after training (T2DN 3.4 ± 2.6 vs. SD 45.0 ± 1.7%). These findings indicate that T2DN is a new type II diabetic rat model. Elderly T2DN rat is associated with an impaired autoregulation of CBF, glial activation and inflammation which may contribute to the development of cognitive impairment and AD.


Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P244

Utility or Futility of Echocardiography in Determining Cardiac Causes of CVA
Hamza A Lodhi, Baptist Memorial Hosp, Southaven, MS; Hasan Shafiq, Aureus Univ Sch of Med, Oranjestad, Aruba; Asim Mushtaq, Cookeville Medical Ctr, Cookeville, TN; Muhammad H Khan, Saint Luke's Medical Ctr, Houston, TX; Ehtesham Haq, Univ Of Alabama, Mobil, AL; Ali Shafiq, Saint Luke's Mid America Heart Inst, Kansas City, MO

Background: Current guidelines recommend that all patients who are suspected to have a cardio-embolic cause for their cerebral vascular accident (CVA) should receive an echocardiogram. However, there are no clear risk stratification tools to help clinicians identify patients at high risk for having cardio-embolic stroke. As a result, echocardiograms appear to be over utilized and may have a low yield in routine clinical practice.

Methods: In our single center study we included patients who were >18 years old and had been admitted from November 2015 to February 2016 to our hospital with an admission diagnosis of Cerebral Vascular Accident (CVA) using ICD-9 code 434.91. Multivariable logistic regression was used to identify factors associated with a greater likelihood of having an echocardiogram ordered as a part of the diagnostic workup.

Results: Among 347 patients who were admitted with a diagnosis of CVA, echocardiogram was ordered in 259 (74.6%) patients. In patients who underwent echocardiography, only 3/259 (0.01%) had abnormal findings (e.g., Patent foramen ovale or intracardiac vegetations) that might suggest a cardio-embolic source for their CVA. In the adjusted analyses we found that factors like age, previous history of CVA, findings of atrial fibrillation on electrocardiogram (p-values > 0.05), were not significantly associated with the decision for ordering echocardiograms.

Conclusions: Our study shows a low yield of echocardiography to determine an embolic cause in patients who were admitted to the hospital with diagnosis of CVA. Furthermore, no patient-level variables were associated with the likelihood of having an echocardiogram ordered. This may suggest that providers’ decisions to order echocardiograms is random rather than systematic, which itself stems from lack of clear recommendations for clinicians in when to order this test. More clear guidelines in this matter will be helpful to ensure appropriate utilization of this modality in CVA patients.


Funding: No

Funding Component: P245

Contraindications and Exclusion Criteria in Guidelines for Rt-pa in Acute Ischemic Stroke: Can the New Aha/asa Guideline Expand the Use of Rt-pa?

Thomas I Nathaniel, Jessica-Ashley Williams, Brian Fazzone, Sara Yi, Gabrielle Morris, Leigh-Ann Black, Megan Fredwall, Clay Staford, Alyssa Adkins, Shuler Polk, Univ of South Carolina Sch of Med Greenville, Greenville, SC

Background: Most of the original exclusion criteria for recombinant tissue plasminogen activator (rt-PA) from NINDS trial, European, and FDA contribute to the low rt-PA use because of their stringent criteria. The new AHA/ASA guideline suggests that by relaxing the already existing criteria the use of rt-PA could be increased from its current use. The impact of the new AHA/ASA guideline in expanding the use, outcomes and benefits of rt-PA is not clear. We determined these issues in the current study.

Methods: We characterized absolute and relative contraindications to rt-PA using a large sample size collected between 2010 and 2013
in a stroke registry. We analyzed both imaging and rt-PA data to determine whether there would have been significant increase in use, outcomes and benefit of rt-PA if the AHA/ASA guideline was used instead of the old FDA package insert of Alterplase (Activase).

**Results:** A total of 663 ischemic stroke patients were eligible to receive rt-PA. Out of the 663, 241 received rt-PA and 422 did not. We identified specific differences in outcomes and contributing factors to contraindications at the level of individual patients and stroke population. We observed clinical conditions of similar exclusion criteria but with differential stroke deficits in both AHA/ASA guideline and the old FDA package insert of Alterplase (Activase).

**Conclusion.** Although the new AHA guideline may serve the purpose of helping to expand the safe and judicious use of alteplase after stroke, there are specific clinical factors that are necessary to achieve the goal of the new AHA/ASA guideline.


Funding: No

Funding Component: P246

**Targeting 20-Hydroxyeicosatetraenoic Acid in Rofecoxib-induced Cerebrovascular Damage**

Mong-Heng Wang, Jing Weng, Adviye Ergul, Augusta Univ, Augusta, GA

**Background:** Coxibs, selective cyclooxygenase-2 (COX-2) inhibitors, significantly improved the quality of life of millions of individuals affected by pain and inflammation. Unfortunately, increased risk of stroke with the use of coxibs limited their clinical usefulness. The goal of this study is to investigate the role of 20-hydroxyeicosatetraenoic acid (20-HETE) metabolism by COX-2 in rofecoxib-induced cerebrovascular damage. We treated MC38 mice (1.4 x 10^8 MC38 cells/mouse) with rofecoxib (50 mg/L) + HET0016 (20-HETE blocker; 5 mg/kg/day, i.p.), rofecoxib, or vehicle for 3 weeks. We then subjected these MC38 and control mice to thromboembolic stroke. Finally, we treated B6 mice with 20-OH PGE_2 (250 ng/h; osmotic pump) and vehicle for 7 days, and then subjected them to ischemic stroke.

**Results:** 1). Rofecoxib significantly reduced MC38 tumor size (from 693 ± 106 to 329 ± 40 mm^3, P < 0.05). Within its therapeutic dose, rofecoxib selectively increased circulating 20-HETE levels (100 ± 18 vs. 150 ± 11%, P < 0.05), which was reversed by HET0016 (150 ± 11 vs. 100 ± 16%, P < 0.05). We did not find significant change on cyp4a (20-HETE synthesizing enzymes) expression in the brain microvessels after rofecoxib treatment. 2). A major prostaglandin (PG) metabolite, which is 20-OH PGE_2, was generated when we incubated purified COX-2 with 20-HETE (5 uM). Strikingly, rofecoxib (1 uM) inhibited 20-OH PGE_2 synthesis by 71% (from 100 ± 16 to 29 ± 8%, P < 0.05). 3). Hemorrhagic transformation (HT) was greater in rofecoxib group (4/5 mice showing bleeding), a vascular injury that was prevented by co-treatment with HET0016 (0/6 mice showing bleeding). 4). 20-OH PGE_2 supplementation reduced both infarct size (45 ± 10 vs. 17 ± 8% contralateral hemisphere, P < 0.05) and neurological deficits (3.6 ± 0.5 vs. 2 ± 1, P < 0.05). **Conclusion:** 1) Rofecoxib increases circulating levels of 20-HETE, which exacerbates stroke damage. 2) COX-2 is the key player in regulating 20-HETE metabolism. 3) 20-HETE blockade prevents HT induced by rofecoxib. 4) 20-OH PGE_2 exerts cerebroprotective after ischemic stroke. In summary, these results suggest that both 20-HETE blockade and 20-OH PGE_2 supplementation are new approaches to ameliorate coxib-induced cerebrovascular damage and adverse stroke outcomes.
M. Wang: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA Grant-in-Aid (AHASE00090). J. Weng: None. A. Ergul: None.

Funding: No
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P247

Non-dipping Ambulatory Blood Pressure in Non-hypertensive Individuals

Cynthia Cheng, Scott Keith, Thomas Jefferson Univ, Philadelphia, PA

Non-dipping predicts increased cardiovascular risk in hypertension. However, the mechanisms leading to non-dipping remain unknown. Also, non-dipping in normotension and prehypertension is less well-described. The study objective was to determine the prevalence of non-dipping, and the association of non-dipping with other cardiovascular risk factors, in non-hypertensive young adults. The study cohort included 176 non-hypertensive, non-diabetic individuals, mean age 27 (Table 1). Office and 24-hour ambulatory BP were measured, along with fasting adipokine levels, insulin sensitivity (QUICKI) and measures of vascular stiffness: Ambulatory arterial stiffness index (AASI) and pulse pressure. The distribution of dipping patterns was 58% dippers, 35% non-dippers, 5% extreme dipping, and 2% reverse dipping. CRP, IL6, and AASI are significantly associated with non-dipping (Table 2).

Non-dipping in common in young non-hypertensive adults. Inflammation and vascular stiffness may be potential mechanisms explaining the increased cardiovascular risk associated with non-dipping.
It has been suggested that aortic BP could be a better predictor of target organ damage (TOD) than peripheral BP. However, studies examining such relationship by using ABPM are scarce. We aimed to examine such relationship in a cohort of hypertensive patients. A total of 208 patients (34% women; mean age: 57±12 years, 90% on antihypertensive treatment) were recruited from 4 hospital clinics in the area of Barcelona, Spain. The presence of TOD was determined if one of the following were present: cardiac, defined as the presence of LVH (LVMI ≥ 115 g/m² in men or ≥ 95 g/m² in women); renal, defined as the presence of either albuminuria (UAE ≥ 30 mg/g) or reduced eGFR (< 60 ml/min/1.73 m², by CKD-EPI formula), or both; vascular: aortic PWV ≥ 10 m/s. Clinic (mean of 4 measurements) and 24-h brachial and aortic BP were determined by the use of the portable automated Mobil-O-Graph device. LVH was present in 37%, albuminuria (17%), reduced eGFR (14%), or both were present in 26%, and elevated aortic PWV in 18%. Overall, 51% of patients presented TOD in at least one organ, whereas 6.3% have all renal, cardiac, and vascular alterations. After adjustments for age, gender, and the use of antihypertensive treatment, odds ratios for clinic, 24-hour, daytime, and nighttime brachial and aortic SBP and pulse pressure (PP) were all significantly associated with the presence of TOD. Higher odds ratios were observed for PP, compared to SBP, and for 24-hour estimates, compared to clinic, daytime or nighttime values. The OR for 5 mmHg increase in brachial and aortic PP were respectively 1.38 (1.16-1.60) and 1.41 (1.14-1.70). PP amplification or augmentation index (clinic or 24-h-derived) were not significantly associated with the presence of TOD, after adjustments. Considering specific organ alterations, aortic SBP and PP estimates were associated with LVH and aortic PWV, but not with albuminuria or reduced eGFR. Both aortic SBP and PP are associated with the presence of TOD in hypertensive patients. The association is stronger for 24-h PP and for LVH and arterial stiffness, whereas renal alterations show a
weaker association. The use of ABPM including aortic BP measurement can be of interest in predicting cardiovascular alterations in hypertension.


Funding: No
Funding Component: P249

Melanocortin-4 Receptors in Cholinergic Preganglionic Neurons of the Hindbrain and Spinal Cord are Important in Mediating Cardiovascular Responses to Acute Stress

Jussara M do Carmo, Taoling Fang, Sydney P. Moak, Jackson R. Browing, John E. Hall, Univ of Mississippi Medical Center, Jackson, MS

Previous studies suggest that melanocortin-4 receptor (MC4R) activation in areas outside of the hypothalamus may regulate appetite but the role of MC4R in specific neuronal populations of the hindbrain in regulating cardiovascular function are still unknown. We examined the impact of total body MC4R deficiency (LoxTB-MC4R mice) and restoration of MC4R specifically in the brainstem and/or spinal cord on blood pressure (BP) response to acute stress. We selectively rescued MC4R only in preganglionic parasympathetic neurons of the dorsal motor of the vagus (DMV) and in the nucleus tractus solitarius (NTS) (LoxTB-MC4R/Phox2B-cre mice, n=5) or in cholinergic preganglionic neurons of the hindbrain and spinal cord (LoxTB-MC4R/Chat-cre mice, n=4). Mice were implanted with telemetry probes for measurement of mean arterial pressure (MAP) and heart rate (HR). After a 10-day recovery period, MAP and HR were continuously measured for 30 minutes before, during and 30 minutes after an air jet stress test. Acute air jet stress significantly increased MAP by 33±3 in WT mice and 31±2 in LoxTB-MC4R/Chat-cre mice compared to only 18±4 or 20±3 mmHg in LoxTB-MC4R/Phox2B-cre mice and LoxTB-MC4R mice, respectively. HR was increased by 180±20, 110±20, 100±23, and 127±15 bpm in response to air jet stress in WT, LoxTB-MC4R, LoxTB-MC4R/Phox2B and LoxTB-MC4R/Chat-cre mice, respectively. These results indicate that MC4Rs in cholinergic preganglionic neurons of the hindbrain and spinal cord play a key role in the BP responses to acute stress. (NHLBI-PO1HL51971, NIGMS- P20GM104357, and AHA-SDG5680016)


Funding: Yes
Funding Component: P250

MDMouse, a Finger Blood Pressure Monitor, Consistently Underestimates Blood Pressure Compared to an Omron Home Blood Pressure Monitor

David Kountz, Yen-Hong Kuo, Anne Detoro, Meridian Health, Neptune, NJ; Sabrina Luisi, Rutgers Univ, Piscataway, NJ; Ahmad AbuHomoud, Urvish Patel, Meridian Health, Neptune, NJ

Purpose: The aim of our study was to investigate the accuracy of MDMouse, a finger blood pressure device that also serves as a mouse for a computer, compared to an Omron home blood pressure monitor among healthy adults with or without controlled hypertension.

Methods: The study design was a prospective trial of healthy adult volunteers recruited to the Clinical Research Center at Jersey Shore University Medical Center. The study was approved by the Meridian Health Institutional Review Board. After 5 minutes of rest subjects
had their blood pressures measured by MDMouse and an Omron 3 Series Upper Arm Blood Pressure Monitor. Blood pressures were measured in both arms using both devices. Concordance of blood pressure between the finger and arm devices utilizing the same arm within 5 mmHg was considered accurate between devices.

**Results:** Data from 91 patients was available for analysis. Their mean age was 52.6 years; 71% were female; 9% non-Hispanic Black, and 32% hypertensive. Figure 1 provides information on concordance for left arm systolic readings with MDMouse. Overall there was poor correlation between readings, with 69.2% (95% confidence interval [CI]: [58.7%, 78.5%]) of readings for SBP and 71.4% (95% CI: [61.0%, 80.4%]) of readings for DBP were > 5mm Hg different (DBP figure not shown). The majority of both SBP and DBP readings from MDMouse were lower than those from the Omron cuff.

**Conclusions:** Our results demonstrated inaccuracy of readings of a finger blood pressure device compared to an automated cuff measuring blood pressure at a brachial site. At present, MDMouse cannot be recommended to measure blood pressure in healthy adults.

---

**Funding:**

**D. Kountz:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Boehringer Ingelheim, Principal Investigator. **Y. Kuo:** None. **A. Detoro:** None. **S. Luisi:** None. **A. AbuHomoud:** None. **U. Patel:** None.

**Funding Component:** P251

**Association of Microvascular Measures with Brachial and Central Pressures in Young Non-hypertensive Adults**

**Cynthia Cheng,** Thomas Jefferson Univ, Philadelphia, PA; Raymond Townsend, Julio Chirinos, Univ of Pennsylvania, Philadelphia, PA; Scott Keith, Thomas Jefferson Univ, Philadelphia, PA

Microvascular rarefaction (reduced capillary density) is associated with hypertension. Little is known about capillary rarefaction in young individuals, in whom brachial and central blood pressure (BP) may be quite divergent. Thus, we compared capillary density in young subjects with their office and central BP profiles.

We measured central BP using the SphygmoCor XCEL device, and office BP in 102 healthy young participants (*Table 1*). Capillary density was measured progressively in the nailbed at baseline rest, following ischemic stimulus, and using passive venous congestion. All capillary densities were significantly associated with central DBP and MAP (*Table 2*). Only post-ischemic capillary density was significantly associated with office brachial SBP and MAP (rest and venous densities were marginally associated).

In conclusion, capillary density is consistently and significantly associated with central MAP in young, non-hypertensive individuals. Since MAP is a key determinant of systemic vascular resistance, capillary rarefaction may lead to the future development of hypertension.
C. Cheng: None. R. Townsend: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Fukuda. E. Honoraria; Modest; UpToDate. G. Consultant/Advisory Board; Modest; Medtronic. J. Chirinos: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; American College of Radiology Network, Fukuda Denshi, Bristol-Myers Squibb, Microsoft Research and CVRx Inc., C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Atcor Medical. G. Consultant/Advisory Board; Modest; Bristol-Myers Squibb, OPKO Healthcare, Fukuda Denshi, Microsoft Research, Merck, AstraZeneca/Fibrogen and Vital Labs.. S. Keith: None. 

Funding: No
Hypertension Educator: Evidence for an Interdisciplinary Approach

Courtney Doyle-Campbell, Western New England Univ, Springfield, MA

Purpose: Certified Diabetes educators have long been utilized to educate patients with diabetes on effective lifestyle changes and to assist in optimizing evidence based medication. Using the same interprofessional prospective to target HTN would be an effective tool in decreasing CV risk. This study demonstrates the efficacy of a clinical pharmacist in controlling BP through patient education and medication intervention within a level 3 medical home.

Methods: This is a retrospective analysis of patients seen by a clinical pharmacist from 2013 to 2015 in a HTN clinic located in a level 3 medical home. Patients are referred to the clinic by primary care physicians. During the one hour initial consultation the clinical pharmacist reviews the patient’s prescription and over-the-counter medications, recommends medication optimization, and educates the patient on medication, lifestyle and HTN. Recommended medication changes, lab requests and specialty referrals are pended to the physician for approval. The patient has the opportunity to follow-up in the HTN clinic until BP is controlled. Impact of pharmacist intervention was assessed based on number of follow-ups to the clinic.

Results: A significant change was noted in the SBP and DBP in patients seen within the HTN Clinic within the 1st, 2nd and 3rd follow-ups (p<0.05) (table 1). There were 312 medication changes recommended by the pharmacist and approved by the PCP during the study period. The patient has the opportunity to follow-up in the HTN clinic until BP is controlled. Impact of pharmacist intervention was assessed based on number of follow-ups to the clinic.

Conclusion: This study provides evidence that incorporating pharmacists into medical homes can provide BP lowering benefits for patients and provide important preventative care.

C. Doyle-Campbell: None.

Funding: No

Funding Component: P253

Carvedilol Benefits in Hypertensive Cardiomyopathy

Renata Dominguez, Univ Nove de Julho - UNINOVE, Sao Paulo, Brazil; Valeria Costa-Hong, Heart Inst (InCor) do Hosp das Clinicas da Faculdade de Medicina da USP, Sao Paulo, Brazil; Fernanda Consolim-Colombo, Luan Ferretti, Univ Nove de Julho - UNINOVE, Sao Paulo, Brazil; Luiz Bortolotto, Heart Inst (InCor) do Hosp das Clinicas da Faculdade de Medicina da USP, Sao Paulo, Brazil; Brent Egan, Univ of South Carolina-Greenville, Greenville, SC; Heno F Lopes, Heart Inst (InCor) do Hosp das Clinicas da Faculdade de Medicina da USP, Sao Paulo, Brazil

Left ventricle remodeling is a common consequence of uncontrolled hypertension. Hypertension treatment including renin-angiotensin-aldosterone system inhibition and beta-blockers offers cardiac protection. Carvedilol uses in cardiomyopathy from different etiology have shown good results in left ventricle reverse remodeling. There is a lack of information regarding carvedilol use in hypertensive cardiomyopathy. The main objective in this study was to evaluate the use
of carvedilol in hypertensive patients with reduced left ventricle ejection fraction. We evaluate 98 subjects with reduced left ventricle ejection fraction (55 years age, 59 males, 64 white, 34 nonwhite) before and after at least six months of carvedilol use. Clinical data, laboratory tests, and echocardiogram were evaluated at least 6 months before and after carvedilol was added to the treatment. Other causes of cardiomyopathy, including coronary artery disease, were excluded. A hundred percent of patients was taking diuretic and renin-angiotensin-aldosterone system inhibitors. Twenty eight percent were using statins, 19% were using antidiabetic drugs, and 29% were taking digoxin. Blood pressure and heart rate (144/92 mmHg, 84 bpm) decreased significantly after (130/82 mmHg, 70 bpm) carvedilol treatment. Ejection fraction improved in 68.5% patients. Let ventricle diastolic diameter decreased from 62 to 56 mm, left ventricle systolic diameter decreased from 53 to 42 mm, left ventricle mass index decreased from 145 to 129 g/m², left ventricle relative posterior wall increased from 0.32 to 0.36. Carvedilol, in addition to antihypertensive drugs, showed improvement in hemodynamic parameters, and in echocardiographic structural and functional parameters in hypertensive cardiomyopathy patients.

R. Dominguez: None. V. Costa-Hong: None. F. Consolim-Colombo: None. L. Ferretti: None. L. Bortolotto: None. B. Egan: None. H.F. Lopes: A. Employment; Modest; None. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Novartis. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; None. D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Novartis. E. Honoraria; Modest; Novartis. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellect); Modest; None. G. Consultant/Advisory Board; Modest; None. H. Other; Modest; Biolab.

Funding: No
Funding Component: P254

Impact of Socially Influencing Systems on Hypertension Control

Khan Siddiqui, Johns Hopkins, Baltimore, MD; Ross Goglia, Higi SH LLC, Chicago, IL; Aamer Ghaffar, mHealthCoach, Chicago, IL

Background: Socially influencing systems (SIS) have shown to impact behavior change and outcomes in various clinical scenarios. Two aspects of SIS, i.e., Social Competition and Social Recognition are also known to increase engagement in a program. Objective: To evaluate the impact of social influence on hypertension control. Methods: All hypertensive patients that had initial hypertensive reading between July 1, 2015 and September 30, 2015 using an ambulatory BP kiosk (higi Station, higi SH llc) were identified. A random sample of 1,352 patients were identified as controls and 38,885 patients were invited to participate in a challenge to check their BP on a weekly basis. Weekly drawing for $25 gift card was conducted for those that checked their BP that week and a grand prize of $100 was awarded to the user with the most BP readings. Challenge duration was from October 1, 2015 to January 20, 2016. Patients who joined also received weekly email reminders to check their BP as well as coaching tips on how to maintain or improve their BP. Patients invited to join challenge but did not participate were referred to as Invitees, those who participated were referred to as Joiners and those not invited as Control. Results: A total of
1,655 patients participated in the challenge. Analysis of variance indicated a statistically significant difference between Control and Joiners (p=.016) as well as between Invitees and Joiners (p=0.009). Controls’ mean arterial pressure change increased 38.5% during the course of the study, while Invitees’ mean arterial pressure change increased 12.2%, and Joiners’ mean arterial pressure change dropped by almost 45%. **Conclusion:** Social competition and social recognition as implemented in the form of a BP check challenge showed significant reduction in mean arterial pressure. Incorporating socially influencing systems in treatment protocols for hypertension can assure adherence to the program and improve outcomes.


Funding: No
Funding Component: P255

**Addressing Management of Resistant Hypertension: Effect of Online CME**

Jelena Spyropoulos, Charles Kearns, Medscape LLC, New York, NY

**Introduction:** Clinicians have a less than adequate understanding of how to properly evaluate patients to diagnose treatment-resistant hypertension, and how to use combination therapies and strategies for managing adherence to therapy to optimize outcomes. **Objective:** To determine if an online, video-based continuing medical education (CME) intervention could improve knowledge and competence of cardiologists and primary care physicians (PCPs) in managing patients with resistant hypertension. **Methods:** An online CME activity was developed as a 25-minute roundtable discussion with 3 leading experts on strategies to manage patients with VTE. The activity included a transcript and a downloadable slide deck to reinforce key data. The effects of education were assessed using a linked pre- /post-assessment study design. For all questions combined, the McNemar’s chi-square test was used to assess differences from pre- to post-assessment. Cohen’s d was used to calculate the effect size. **Results:** The change in correct responses from pre- to post-assessment achieved statistical significance for all 4 questions for cardiologists (N=156; d=0.981; P<.001) and PCPs (n=539; d=0.978; P<.001) with a large effect size for both specialty groups (Table). **Conclusion:** The significant improvements observed as a result of participation in this CME intervention demonstrate that well-designed internet-based education can improve knowledge and competence of physicians. However, both cardiologists and PCPs demonstrate a need for further education on the prevalence of resistant hypertension, clinical data, and strategies to address nonadherence to antihypertensive therapy.

<table>
<thead>
<tr>
<th>Question</th>
<th>Pre-assessment</th>
<th>Post-assessment</th>
<th>Change</th>
<th>Effect Size</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of hypertension</td>
<td>65%</td>
<td>75%</td>
<td>10%</td>
<td>0.978</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Nonadherence</td>
<td>52%</td>
<td>40%</td>
<td>12%</td>
<td>0.981</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Strategies for managing</td>
<td>73%</td>
<td>80%</td>
<td>7%</td>
<td>0.978</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Reduction in mean arterial pressure</td>
<td>64%</td>
<td>55%</td>
<td>9%</td>
<td>0.981</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

J. Spyropoulos: None. C. Kearns: None.

Funding: No
Funding Component: P256
Bone Health Predicts Cardiovascular Risk in Adolescents

Deborah L Stewart, GREGORY A HARSHFIELD, Laura Carbone, Augusta Univ, Augusta, GA; Coral Hanevold, Univ of Washington, Seattle, WA

This study examined the effects of mental stress on indices of cardiovascular risk (CVR) in children and adolescents including total bone mineral density (TBMD). The subjects included 272 healthy individuals aged 15-19 years with 143 females and 129 males, including 137 African-Americans and 135 Caucasians. Indices of CVR included: echocardiographic measurements of left ventricular mass adjusted to height^{2.7} (Lvmht^{2.7}), and left ventricular internal dimension during diastole (LVIDd), casual measurements including systolic blood pressure (CSBP) and weight, and SBP during mental stress. Each of these indices of CVR was associated with increased TBMD, as shown in the table. When analyzed separately for males and females, all the associations remained significant in males. However, only weight and stress SBP were significant in females. With respect to ethnicity, the correlations were significant and similar for AAs and Caucasians with the exception of stress SBP for Caucasians which approaches significance. The results of the study suggest a link between bone and the heart. Furthermore, the fact that we observed this link in children and adolescents suggests it is expressed early in life prior to the development of significant heart disease and thus provides opportunity for preventative strategies.


Funding: No

Funding Component: P257

Taking Fresh Aim at Improving Blood Pressure Control

MICHAEL K RAKOTZ, Omar Hasan, American Medical Association, Chicago, IL; Eduardo Sanchez, American Heart Association, Dallas, TX; Greg Wozniak, American Medical Association, Chicago, IL

Nearly 70 million adults in the United States have hypertension, and only about 50% of those adults have a blood pressure reading below 140/90 mm Hg. Target: BP is a national initiative co-led by the American Heart Association and American Medical Association aimed at improving blood pressure control nationally to reduce the number of Americans who suffer the consequences of high blood pressure including myocardial infarctions, heart failure, strokes, and chronic kidney disease. Target: BP will equip physician practices and health care systems with the resources and technical assistance to achieve a minimum 70% blood pressure control rate with a target of reaching 80% or higher. Target: BP is a multi-faceted, evidence-based, data-driven quality improvement initiative that leverages the AMAs membership and strategic focus on improving health outcomes and the AHAs success in disseminating large scale improvement initiatives like Get With the Guidelines - Stroke, an effective cerebrovascular quality improvement and recognition program. This initiative offers participating healthcare systems, ambulatory clinical practices and individual clinicians the following:

- A mass communication campaign to raise public awareness about the
importance of controlling high blood pressure

• A quality improvement program and relevant tools to help clinicians and care teams implement the latest hypertension guidelines and improve management and treatment of patients with hypertension, including check lists, treatment algorithms, protocols and fact sheets

• Opportunities to contribute data for tracking blood pressure control rates and benchmarking against other participants regionally and nationally

• Opportunities to be recognized formally for achieving a 70% blood pressure control rate, improving blood pressure control, reducing therapeutic inertia and using self-measured blood pressure monitoring

• Access to a national advisory group of experts in improving blood pressure control

Since its initiation in November 2015, over 250 healthcare systems and clinics have committed to participate in Target: BP.

M.K. Rakotz: None. O. Hasan: None. E. Sanchez: None. G. Wozniak: None.

Funding: No

Funding Component: P258

**Antihypertensive Medication Nonadherence Among Hispanic Adults by Healthcare Access and Acculturation Characteristics - Estilos Survey, 2015**

Carma Ayala, CDC/NCCDPHP/DACH, Atlanta, ID; Xin (Cindy) Tong, CDC/NCCDPHP/DACH, Atlanta, GA; Carol Ochoa, Emory Rollins Sch of Public Health, Atlanta, GA; Carla Mercado, CDC/NCCDPHP/DACH, Atlanta, GA; Jing Fang, CDC/NCCDPHP/DACH, Atlanta, ID

Medication adherence for hypertensive adults (HTNs) is an important factor in achieving and maintaining blood pressure control, as it has been associated with better health outcomes and lower costs of medical care. We assessed the differences in the prevalence of self-reported antihypertensive medication use and adherence among Hispanic HTNs by selected characteristics. Methods: ESTILOS is an online panel for health survey data of Hispanic adults in the USA. In 2015, 1,000 adults completed the survey with a response rate of 29%. The resulting data were weighted using 8 factors: gender, age, household income, household size, education, census region, country of origin, and acculturation (based on years living in the USA, language spoken at home, cultural self-identification, and use of Spanish language). The differences among characteristics for prevalence + standard error of medication use and adherence were compared by using $\chi^2$ statistics. Results: The prevalence of hypertension was 27.5±2.7%. The prevalence of antihypertensive medication use in HTNs was 67.9+4.6%) overall and was higher among those ≥55 years of age (95.2±2.1%; $P<$0.0001), retirees (92.3±4.0%; $P=0.0002), who had healthcare coverage (72.5±4.8%; $P=0.02) and had a primary care physician (PCP) (71.9+4.9%; $P=0.03), did not have any reported cost barriers to seeing their PCP within past 12 months (71.9±5.3%; $P=0.001), and took 3-4 lifestyle actions (79.7±5.4%; $P=0.015) than <3 lifestyle actions). Among HTNs taking antihypertensives, 24.0±7.0% reported missing or skipping medication doses. HTNs aged <55 years or who could not see a PCP within the past year due to cost barriers, were more likely to miss or skip doses of antihypertensive medication (<55=54.1±13.4%) vs >55=11.5±5.3%; $P=0.03$ and could not see PCP=45.2±12.1% vs 12.0±5.2%; 0.01, respectively). Conclusion: Poor antihypertensive medication adherence among Hispanics with hypertension was associated
with younger age groups and those who reported barriers to engaging with a PCP. Public health practitioners and clinicians can utilize this information to support their programs and can tailor strategies for improved outcomes.

C. Ayala: None. X. Tong: None. C. Ochoa: None. C. Mercado: None. J. Fang: None.

Funding: No
Funding Component: P259

Social and Biological Correlates of Elevated Blood Pressure in Afro-Caribbean Youth: Effect of Individual Risk Factors and Risk Factor Clustering

Trevor S Ferguson, Novie O Younger-Coleman, Marshall K Tulloch-Reid, Jennifer M Knight-Madden, Maureen E Samms-Vaughan, Deanna E Ashley, Rainford J Wilks, The Univ of the West Indies, Kingston, Jamaica

Background: We aimed to estimate the relative risk for elevated blood pressure (BP ≥ 120/80 mmHg) for cardiovascular disease (CVD) risk factors among Afro-Caribbean youth in Jamaica and to evaluate the association between clustering of risk factors and elevated BP.

Methods: We analysed data from 898 young adults, 18-20 years old (409 males; 489 females) from the Jamaica 1986 Birth Cohort Study. BP was measured with a mercury sphygmomanometer after the participant had been seated for 5 minutes. Anthropometric measurements were done and venous blood obtained to measure fasting glucose, lipids and insulin. Data on socioeconomic status (SES) were obtained via questionnaire. CVD risk factor status was defined using standard cut-points or the upper quintile of the distribution. Insulin resistance was estimated using the Homeostasis Model Assessment (HOMA-IR). Relative risks were computed using odds ratios (OR) from logistic regression models. Results: Prevalence of elevated BP was 30% among males and 13% among females (p<0.001). In multivariable logistic regression models, modifiable risk factors independently associated with elevated BP among males were: central obesity (OR 4.7, 95%CI 1.6 - 13.7), high glucose (OR 1.9, CI 1.1 - 3.1) and high HOMA-IR (OR 2.33, CI 1.1 - 5.1); among females associated factors were: high triglycerides (OR 2.2, CI 1.1 - 4.1), high HOMA-IR (OR 2.4, CI 1.1 - 5.0) and SES (OR 3.6, CI 1.03 - 12.8 [moderate vs high household possessions]; OR 1.87 CI 0.5 - 7.2 [low vs high]). Among males, having any one of central obesity, high glucose, high triglycerides or high HOMA-IR was associated with a two-fold increase in the odds of elevated BP, while having three or four factors was associated with a seven-fold increase in the odds. Among females, having any one of the four factors above was associated with two-fold higher odds, while having three or four factors was associated with a three-fold higher odds.

Conclusion: Factors associated with elevated BP among Jamaican young adults include measures of obesity and insulin resistance, with significant differences by sex. Lower SES was associated with elevated BP among females. Clustering of risk factors was associated with markedly higher odds of elevated BP among males, but less so among females.


Funding: No
Funding Component: P260

Systolic Blood Pressure and Lifestyle Contributes to Development of a Risk Score for Incident Atrial Fibrillation in the Japanese General Population: The Suita Study
Yoshihiro Kokubo, Makoto Watanabe, Aya Higashiyama, Yoko M. Nakao, Fumiaki Nakamura, Kunihiro Nishimura, Misa Takegami, Kengo Kusano, Yoshihiro Miyamoto, Natl Cerebral and Cardiovascular Ctr, Suita, Japan

Purpose: Atrial fibrillation (AF) is a strong risk factor for mortality and stroke. However, there is no risk score for incident AF in non-Westerners. We developed and validated a risk score for AF incidence in a Japanese general population.

Methods: A total of 6,864 participants (30-79 years old) initially free of AF have been prospectively followed up for incident AF since 1989. Standard 12-lead ECGs were obtained from all subjects in the supine position. Each record was coded independently by 2 well-trained physicians. Participants were diagnosed with AF when AF or atrial flutter was present on ECGs from a biannual routine health examination or when AF was indicated as a present illness by either annual questionnaires responses or participants' medical records during follow-up. The end point of the follow-up period was whichever of the following options occurred first: the date of the first AF event, date of the last health examination and medical records, and December, 2015. Cox proportional hazard ratios were analyzed after adjusting for cardiovascular risk factors at baseline. The model discrimination was evaluated by the area under the receiver operating characteristic curve.

Results: In 95,180 person-years of follow-up, 311 incident AF events occurred. Age, sex, overweight (including obesity), valvular disease (including heart murmur), systolic blood pressure, ischemic heart disease, current smoking, and excessive drinking (4 units/day or more of alcohol) were associated with incident AF (C-statistic 0.72; 95% confidence intervals, 0.70-0.74). The risk scores for participants were 0, 4, 8, and 11 in their 30s-40s, 50s, 60s, and 70s; 2 in men; 1 and 2 with current smoking and excessive drinking; 2 and 3 in their systolic prehypertension with overweight and systolic hypertension with normal weight or overweight; and 2 and 3 with ischemic heart disease and valvular disease, respectively. Predicted 10-year risk of AF was similar to observed risks. Individuals scoring of 5 and 15 points, for example, had 1.7% and 9.6% predicted probability of developing AF in 10 years, respectively.

Conclusion: We developed a risk score to predict 10-year incident AF risk using variables that are easily available in healthy examination in Japan.


Funding: No

Funding Component: P261

Intensive Systolic Blood Pressure Lowering Will Prevent Over 100,000 Deaths Annually

Holly Kramer, Loyola Medical Ctr, Maywood, IL; Adam Bress, Univ of Utah, Salt Lake City, UT; Srinivasan Beddhu, Loyola Medical Ctr, Maywood, IL; Paul Muntner, Univ of Alabama, Birmingham, AL; Richard S. Cooper, Loyola Medical Ctr, Maywood, IL

Background: The Systolic Blood Pressure Intervention Trial (SPRINT) trial randomized 9,361 adults aged ≥50 years at high cardiovascular disease (CVD) risk without diabetes or stroke to intensive systolic blood pressure (SBP) lowering (≤120 mmHg) or standard SBP lowering (≤140 mmHg). After a median follow up of 3.26 years, all-cause mortality was 27% (95% CI 40%, 10%) lower with intensive SBP lowering. We estimated the
potential number of prevented deaths with intensive SBP lowering in the U.S. population meeting SPRINT criteria. **Methods:** SPRINT eligibility criteria were applied to the National Health and Nutrition Examination Survey 1999-2006, a representative survey of the U.S. population, linked with the mortality data through December 2011. Eligibility included (1) age ≥50 years with (2) SBP 130-180 mmHg depending on number of antihypertensive classes being taken, and (3) presence of ≥1 CVD risk conditions (history of coronary heart disease, estimated glomerular filtration rate (eGFR) 20 to 59 ml/min/1.73 m², 10-year Framingham risk score ≥15%, or age ≥75 years). Adults with diabetes, stroke history, >1 g/day proteinuria, heart failure, on dialysis, or eGFR<20 ml/min/1.73m² were excluded. Annual mortality rates for adults meeting SPRINT criteria were calculated using Kaplan-Meier methods and the expected reduction in mortality rates with intensive SBP lowering in SPRINT was used to determine the number of potential deaths prevented. Analyses accounted for the complex survey design. **Results:** An estimated 18.1 million U.S. adults met SPRINT criteria with 7.4 million taking blood pressure lowering medications. The mean age was 68.6 years and 83.2% and 7.4% were non-Hispanic white and non-Hispanic black, respectively. The annual mortality rate was 2.2% (95% CI 1.9%, 2.5%) and intensive SBP lowering was projected to prevent 107,453 deaths per year (95% CI 45,374 to 139,490). Among adults with SBP ≥145 mmHg, the annual mortality rate was 2.5% (95% CI 2.1%, 3.0%) and intensive SBP lowering was projected to prevent 60,908 deaths per year (95% CI 26,455 to 76,792). **Conclusions:** We project intensive SBP lowering could prevent over 100,000 deaths per year of intensive treatment.

H. Kramer: None. A. Bress: None. S. Beddhu: None. P. Muntner: None. R.S. Cooper: None.

**Funding:** No

**Funding Component:** P262

**Spot Urine Sodium to Potassium Ratio Predicts Increasing Blood Pressure Levels in a General Population: The Nagahama Study**

Yasuharu Tabara, Yoshimitsu Takahashi, Takeo Nakayama, Fumihiko Matsuda, Kyoto Univ, Kyoto, Japan

Excessive salt intake is a risk factor for hypertension. The most reliable method for estimating daily salt intake is measurement of 24-h urinary sodium excretion, while it is inconvenient. Sodium-to-potassium ratio (Na/K) of a urine sample is another index of salt loading. We previously reported that a simple measure of spot urine Na/K might be a representative of salt loading in a cross-sectional setting. Here, we conducted a longitudinal study aiming to clarify a prognostic significance of spot urine Na/K for increasing blood pressure (BP) levels. Study subjects consists of 9,769 general individuals. Among them, individuals whose baseline Na/K was available (n=9,328), who were normotensive at baseline (n=6,392), and who participated in the follow-up measurement (n=5,209) were included in this analysis (51.8±12.9 years old, male: 29.2%). Mean follow-up duration was 5.0±0.5 years. Mean Na/K at baseline was 3.1±1.7, and showed step-wise increase with BP levels (optimal: 3.0±1.6, normal: 3.3±1.8, high normal: 3.4±1.8, P<0.001). Other major factors that were significantly associated with Na/K was fasting time (r=-0.220, P<0.001), and CKD (CKD (n=694): 2.7±1.6, control: 3.2±1.7, P<0.001). Mean SBP was significantly increased during follow-up period (baseline: 116±12, follow-up: 119±15 mmHg), and 805 individuals (15.5%) were newly diagnosed as hypertension (HT). These individuals were significantly older (HT: 60.3±9.9, NT: 50.3±12.8 years), were frequently
male (36.4%, 27.9%), and had higher SBP (127±9, 115±11 mmHg) at baseline (P<0.001). In contrast, baseline spot urine Na/K was slightly lower in individuals who developed HT (3.0±1.6, 3.1±1.8, P=0.013), while that measured at follow-up investigation was oppositely higher in hypertensives (3.1±1.8, 2.8±1.5, P<0.001).

Multiple linear regression analysis adjusted for the covariates identified baseline Na/K (β=0.108, P<0.001) and changes in Na/K during follow-up period (β=0.222, P<0.001) as independent determinants for future SBP levels. Higher spot urine Na/K, as well as increases in the Na/K levels, was significant determinant for future BP levels. The apparently lower baseline Na/K levels in individuals who developed HT might be due to reverse causality.


Funding: No
Funding Component: P263

Identification and Analyses of MiRNAs And LncRNAs Influencing Hypertension in Dahl Salt-sensitive Rats

Kosuke Endo, Naoharu Iwai, Natl Cerebral and Cardiovascular Ctr, Osaka, Japan

Background: Noncoding RNAs (ncRNAs), including microRNA (miRNA) and long noncoding RNA (lncRNA), are functional and non-protein coding RNA molecules. miRNAs are small ncRNAs of 18-23 nucleotides, whereas lncRNAs are longer than 200 nucleotides and were recently recognized as a class of genomic regulatory molecules and reported in various species. Nonetheless, their physiological functions have not been elucidated.

Methods: In order to investigate a possible involvement of ncRNAs in salt-sensitive hypertension, we performed ncRNA sequencing and transcriptome analysis in Dahl salt-sensitive rats and Lewis rats. The pathway analysis were used to understand the biological roles of differently expressed ncRNAs.

Results: Following ncRNA sequencing, 591 transcripts were defined as mature miRNAs and 2636 transcripts as lncRNAs. Of these, 9 miRNA were mapped to candidate chromosomal regions (chromosome 1, 10, and 12), previously reported to be involved in salt-sensitive hypertension. Differential expression was detected for 3 of these miRNAs (miR-150-5p, miR-7a-5p, miR-21-5p) (fold up/down ≥ 1.2, q < 0.05). However, only one lncRNA (XLOC_081666) encoded by the candidate region of chromosome 1 showed no difference.

In contrast, 10 miRNAs (miR-1b, miR-221-3p, miR-300-3p, miR-411-5p, miR-184, miR-205, miR-129-5p, miR-741-3p, miR-379-5p, miR-138-5p) and 5 lncRNAs (XLOC_102471, XLOC_047839, XLOC_253638, XLOC_778532, XLOC_1029112), located in other regions, showed significant differential expression (fold up/down ≥ 2.0, q < 0.05). The pathway analysis showed that Wnt and MAPK signaling pathways are associated with the targets of detected miRNAs and metabolic pathways are associated with the possible targets of detected lncRNAs.

Although expression levels of target mRNAs in these pathways showed no differences between Dahl salt-sensitive and Lewis rats, it is likely that effects of a single ncRNA on mRNA expression are subtle and may exhibit different response to salt intake.

Conclusions: Our results demonstrate the probable implication of miRNAs and lncRNAs in salt-sensitive hypertension. This new finding may contribute to the exploration of candidate factors involved in the hypertension mechanism.

K. Endo: None. N. Iwai: None.

Funding: No
Funding Component: P264
DNA Methylation and Differences in Blood Pressure Levels in Monozygotic Twins

Srividya Kidambi, Yingchuan Li, Pengyuan Liu, Michelle L leittl, Gerard Coly, Allen W Cowley Jr., Theodore A Kotchen, Yong Liu, Cheryl E Rockwell, Kelly L Klump, S Alexandra Burt, Eric P Gernaat, Mary F Donohue, Supratik Rayamajhi, Ralph E Watson, David L Mattson, Mingyu Liang, Medical Coll of Wisconsin, Milwaukee, WI

Background: The development of common forms of hypertension (HTN) involves both genetic and environmental factors. Methylation changes (one of the epigenetic modifications) of DNA may play a role in the regulation of BP and development of HTN, and may result from interaction of specific genes with the environment. The current study investigates the relationship between genome-wide changes in DNA methylation in T-lymphocytes and BP level differences among monozygotic twins, who have identical DNA sequences. Methods: In a preliminary study, we recruited 24 pairs of monozygotic twins (60% women) with a mean age of 44 ± 10 years. Zygosity was determined via self-report or participants’ responses to a standard zygosity questionnaire. BPs were measured in triplicate after 5 minutes of rest at one minute intervals and averaged. Anthropometrics measurements were obtained along with blood for isolation of T-lymphocytes. DNA from T-lymphocytes was used to perform reduced representation bisulfite sequencing (RRBS) to measure methylation levels at single-base resolution in these subjects. Average differences in systolic (SBP) and diastolic (DBP) BPs were used as continuous variables for these analyses. Results: Mean SBP was 124 ± 15 (range: 97-174) mm Hg and mean DBP was 78 ± 11 (range: 49-106) mm Hg. Average differences in SBP and DBP among the co-twins (members of a twin pair) were 9 ± 10 (range: 0-40) mm Hg and 8 ± 6 (range: 0-26) mm Hg respectively. Average BMI was 29 ± 8 kg/m². We observed that an average of 3063 (range: 757-7250) CpG islands were differentially methylated (DMRs) between co-twins. Among these DMRs, 10 were associated with average SBP difference and 5 were associated with average DBP difference at a significance level of p < 0.001. DMRs showing the strongest association (unadjusted p < 10⁻⁴) between the co-twins were located in the transcriptional start site (TSS) of genes: CDC26 (cell division cycle protein) and NR2F6 (nuclear receptor) for average SBP difference and LEPRE1 (propyl 3 hydroxylase protein) for average DBP difference. Conclusions: BP differences between monozygotic co-twins, which most likely result from environmental factors, appear to be associated with differences in DNA methylation in T lymphocytes.


Funding: Yes
Funding Component: National Center P265

Blood Pressure Control is Better and Less Expensive in Chronic Kidney Disease When Associated Metabolic Acidosis is Treated with Fruits and Vegetables Rather Than Sodium Bicarbonate

Nimrit Goraya, Baylor Scott and White Health, Temple, TX; Jan Simoni, Texas Tech Univ Health Sciences Ctr, Lubbock, TX; Jessica Pruszynski, Pin Xiang, Donald Wesson, Baylor Scott and White Health, Temple, TX
**Background:** Both sodium bicarbonate (NaHCO₃) and base-producing fruits and vegetables (F+V) improve metabolic acidosis in chronic kidney disease (CKD) and appear to provide similar levels of kidney protection. Because F+V themselves reduce blood pressure, we examined if treatment of metabolic acidosis in CKD with F+V was associated with improved blood pressure control, using fewer anti-hypertensive drugs, and thereby with lower cost of hypertension management.

**Methods:** We randomized 108 subjects with CKD stage 3 eGFR (30-59 ml/min) and metabolic acidosis as follows: F+V (n=36) added to reduce dietary potential renal acid load (PRAL) 50%, oral NaHCO₃ (HCO₃, n=36) to reduce PRAL 50%, or no alkali (Usual Care, n=36). All were treated toward systolic blood pressure (SBP) <130 mm Hg with regimens including ACE inhibition and followed 5 years.

**Results:** Entry SBP and initial doses of 5 formulary anti-hypertensive drugs most commonly used for blood pressure control in CKD were not different among the 3 groups. At 5 years, SBP was lower in F+V (125±5 mm Hg) than both HCO₃ and Usual Care (135±5 and 134±5 mm Hg, respectively, p<0.01 vs. F+V for each). Daily doses for the following drugs at year 5 were lower in F+V than HCO₃ and Usual Care: Enalapril (8.3±2.4 vs. 11.1±3.6 and 11.7±4.8, mg/day, respectively, p<0.01), Diltiazem (1.7±7.0 vs. 145.8±36.0 and 153.3±35.7, mg/day, p<0.01), Clonidine (0.14±0.20 vs. 0.65±0.15 and 0.63±0.16, mg/day, p<0.01), Atenolol (0 vs. 6.25±15.1 and 6.25±15.1 mg/day, p<0.02) but there was no difference among groups in the year 5 dose of hydrochlorothiazide (16.1±9.9 vs. 21.9±16.2 and 21.5±16.3 mg/day, p=0.27). Five-year drug cost of hypertension management was less in F+V ($79,760) than both HCO₃ ($155,372) and Usual Care ($152,305).

**Conclusions:** Treating metabolic acidosis in CKD patients with F+V but not NaHCO₃ was associated with lower SBP, use of fewer and lower doses of anti-hypertensive drugs, and lower group cost of hypertension management. The data support that clinicians consider these adjunctive benefits of F+V on hypertension management when recommending treatment strategies for metabolic acidosis in CKD.


Funding: No

Funding Component: P266

**Thromboxane-prostanoid Receptors (tp-rs) Mediate Hypertension, H2o2 Generation and Impaired Renal Afferent Arteriolar Myogenic Responses Leading to Nephropathy in Doca/salt Mice**

Christopher S Wilcox, Lingli Li, En Yin Lai, Adam Hosszu, William J Welch, Georgetown Univ, Washington, DC

Background: DOCA/uninephrectomy/high salt (DOCA) is a model of hypertensive nephropathy. Afferent arteriolar myogenic responses prevent hypertensive renal barotrauma but myogenic tone is blocked by vascular generation of H₂O₂. Since thromboxane-prostanoid receptors (TP-Rs) generate H₂O₂, we tested the hypothesis that they mediate hypertensive nephropathy.

**Methods:** DOCA and Sham TP-R +/+ and -/- mice (n=6/group) were studied at 2 weeks and myogenic responses recorded from the diameter of perfused single afferent arterioles studied in a bath preparation during increased perfusion pressure (40 to 80 mmHg). Results: DOCA treatment in TP-R +/+ mice increased (p<0.001) 24-hour excretion of H₂O₂ (45 ± 3 vs 220 ± 15 nmol), TxB₂ (4 ± 2 vs 29 ± 4 pmol) and albumin (20 ± 5 vs 270 ± 20 mg) and increased MAP by 35 ± 5 mmHg. However, all effects of DOCA were prevented in TP-R -/- mice. Sham treatment had no effect in TPR +/- mice.
Myogenic responses were severely impaired in DOCA vs sham WT mice (Δ diameter: -4 ± 1 vs -8 ± 1%; p< 0.005). Myogenic responses also were reduced by incubation of arterioles with 10^{-10} mol·l^{-1} of the TP-R mimetic, U-46,619 vs vehicle added to the bath for 10 minutes (Δ diameter: -7 ± 1 vs -10 ± 1%; p<0.01) and in WT mice infused for 3 days with U-46,619 (500 ng·kg^{-1}·d^{-1} x 3) vs vehicle (Δ diameter: -3 ± 1 vs -10 ± 1%; p<0.005). Conclusion: Hypertensive nephropathy is dependent on TP-Rs that mediate the increase in H_{2}O_{2} and blood pressure and likely the impaired myogenic responses that expose the kidney to barotrauma.

C.S. Wilcox: None. L. Li: None. E. Lai: None. A. Hosszu: None. W.J. Welch: None.

Funding: No
Funding Component: P267

Sex Differences in Cyp450 Activity and the Development of Hypertension and Renal Injury in the Dahl S Rat

Wenjie Wu, Sydney Murphy, Univ of Mississippi Medical Ctr, Jackson, MS

It is well documented that a sexual dimorphism exists in the regulation of blood pressure in both the human population as well as experimental animal models, however evidence of a sex difference is lacking in the Dahl S rat. Thus, we hypothesize that alterations in CYP450 expression and 20-HETE production contribute to the progression of renal injury in Dahl S rats. Consistent with what we have previously reported, no difference was noted in the blood pressure of male or female Dahl SSJr rat (213.8±12 vs 196.8±13 mmHg, ns) following 4 weeks of a high salt diet (8%NaCl). However, proteinuria (148±25 vs 355±22 mg/day, p<0.05) and renal injury (1.9±0.01 vs 2.5±0.2) were lower in female relative to male rats. In addition, GFR was significantly reduced in male vs female rats (392.4±89 vs 829.5±98 µl/min/g, p<0.05) following at high salt challenge. Renal cortical (11.3±16 vs 20.9±2.8 pmol/min/mg, p<0.05) and outer medullary (19.4±3 vs 6.9±1.8 pmol/min/mg, p<0.05) 20-HETE production was elevated in female versus male rats. Furthermore, renal vascular 20-HETE production was elevated in the renal vessels compared to males (0.53±0.23 vs 3.2±1.2 pmol/min/mg, p<0.05). Thus, alterations in the production of renal eicosanoids may contribute to the delay in renal injury in females relative to male Dahl SSJr rats. AHA 14SDG20160020

W. Wu: None. S. Murphy: None.

Funding: Yes
Funding Component: National Center
P268

The Effects of a Slow Pressor Dose of Angiotensin II on Sodium Transporters Expression in the Kidney Cortex are Independent of PPAR-alpha

Syed J Khundmiri, Howard Univ, Washington, DC; Carolyn M Ecelbarger, Georgetown Univ, Washington, DC; Dexter L Lee, Howard Univ, Washington, DC

A slow pressor dose of Angiotensin II (Ang II) has been shown to increase the expression of sodium transporters in the proximal tubules (NHE3), TALH (NKCC2) and distal nephrons (NCC) of Sprague Dawley rats before an increase in blood pressure. Peroxisome Proliferator Activated Receptor - alpha (PPAR-alpha) has been shown to be involved in pressure natriuresis through changes in sodium transport via ameloride and thiazide-dependent mechanisms. We hypothesized that the changes in expression of the sodium transporters during Ang II hypertension were dependent upon PPAR-alpha expression. To address this
hypothesis, we treated wild-type (WT) and PPAR-alpha knockout (KO) mice with a slow pressor dose of Ang II (400 ng/kg/min) for 12 days. Mean arterial pressure (MAP) was measured by radiotelemetry. Control MAP was not different between WT (110 ± 8 mmHg) and PPAR-alpha KO mice (112 ± 12 mmHg). On day 12 of Ang II, MAP for PPAR-alpha KO (156 ± 16) mice was significantly higher than WT (138 ± 11 mmHg) mice. The expression of NHE3, NHERF1, NKA-α1 subunit, NKCC2, and NCC was detected in kidney cortical homogenates by western blotting. Kidneys were homogenized and 25 μg of supernatant proteins were separated by 10% SDS-PAGE, transferred to nitrocellulose paper, and blotted against antibodies to NHE3, NHERF1, NKA α1 subunit, NKCC2, and NCC. The slow pressor dose of Ang II decreased the expression of NHE3 in WT + Ang II (0.14 ± 0.02 ODU) and PPAR-alpha KO + Ang II (0.10 ± 0.02 ODU), when compared to WT (2.61 ± 0.93 ODU) and PPAR-alpha KO (2.20 ± 0.58 ODU) controls. Ang II-treatment also decreased NKCC2 in both WT (0.31 ± 0.10 ODU) and PPAR-alpha KO (0.22 ± 0.03 ODU). Ang II hypertension caused similar decreases in NCC and NHERF1 expression in WT and PPAR-α KO mice. NKA alpha1 subunit expression was increased during Ang II hypertension in both WT (1.06 ± 0.26 ODU) and PPAR-α KO (1.64 ± 0.26 ODU) mice. Our results suggest that the effects of a slow pressor dose of Ang II on expression of sodium transporters are independent of PPAR-alpha expression. Future studies are needed to determine the effects of decreasing NHE3, NKCC2, NCC and NHERF1 expression in the kidney on urinary salt excretion during a slow pressor dose of Ang II.

S.J. Khundmiri: None. C.M. Ecelbarger: None. D.L. Lee: None.

Funding: Yes
Funding Component: National Center
P269

Sodium Transporter Profile in Mice Lacking AT1A Receptors in the Renal Proximal Tubule


We have reported that mice lacking AT1A receptors (KO) in renal proximal tubule (PT) have 10 mmHg lower baseline BP and less PT fluid reabsorption than wild type (WT). We tested the hypothesis that the lower BP is associated with less abundant renal Na transporters or regulators. Homogenates of renal cortex and medulla (n=6/group) were prepared and 1 and 1/2 protein amounts of each subjected to immunoblot analysis with specific antibodies and quantitated. Results for cortex and medulla, displayed as mean +/- SEM, normalized to mean abundance of WT=1 (*p <0.05), are summarized in figures. In KO vs. WT abundance of PT NHE3, the associated motor myosin VI, the paracellular NaCl transporter claudin 2, and the Na-HCO3 transporter NBCe1 are lower in KO; in the thick ascending limb (TAL) NKCC2 and its associated kinase SPAK are less abundant, and there is a tendency for lower DCT NCC and CCD ENaC in KO. The results support our hypothesis and suggest that KO of PT AT1R reduces transport routes not only in the PT but beyond the PT, in spite of increased volume flow from PT and lower BP.

Funding: Yes
Funding Component: National Center
P269
**Carotid Stenosis: A Frequently Overlooked Etiology of Secondary Hypertension**

J Scott Pannell, Mihir Gupta, Arvin R. Wali, Peter Abraham, Kevin A. Porras, Vincent J. Cheung, Yasaman Alam, Alexander A Khalessi, Univ of California San Diego, San Diego, CA

**Introduction:** Hypertension is one of the most prevalent etiologies for stroke, myocardial infarction, heart failure, retinopathy, and renal failure. Both primary and secondary hypertension can frequently be controlled and end organ damage be prevented by medications alone. However, atherosclerotic diseases resulting in end organ ischemic demand can overwhelm even the most aggressive treatment strategies, which have been demonstrated in cases of renal artery stenosis. However, carotid stenosis is an often-overlooked etiology in secondary hypertension, which can be treated by open surgery or endovascular stenting. **Methods:** In this retrospective study, the medical records of 43 consecutive patients who underwent carotid stenting for symptomatic or severe internal carotid artery stenosis from November of 2013 to December of 2015 were reviewed. All patients either had a prior stroke, TIA, or stenosis of the internal carotid artery measuring greater than 70% by NASCET criteria. All of the patients in the study had a diagnosis of hypertension. The systolic and diastolic blood pressures as well as the anti-hypertensive medication regimen of all of the patients involved were recorded before the surgery, immediately following surgery, and 3 months after the surgery. The average blood pressure reduction was calculated immediately following surgery and at 3 months. **Results:** The average reduction in systolic blood pressures immediately following surgery and at 3 months were 13.86 mmHg and 5.89 mmHg, respectively. The average reduction in diastolic...
blood pressures immediately following surgery and at 3 months were 8.96 mmHg and 1.84 mmHg, respectively. Additionally, the number of anti-hypertensive medications and the dosages decreased in 11 patients, including two of which no longer required anti-hypertensives after stenting. **Conclusion:** Although the treatment of carotid artery disease is typically reserved for patients with cerebrovascular compromise, carotid artery stenting and endarterectomy can be useful adjunctives in the treatment of malignant and uncontrolled hypertension.

**J.S. Pannell:** G. Consultant/Advisory Board; Modest; Codman, Stryker, Microvention. **M. Gupta:** None. **A.R. Wall:** None. **P. Abraham:** None. **K.A. Porras:** None. **V.J. Cheung:** None. **Y. Alam:** None. **A.A. Khalessi:** G. Consultant/Advisory Board; Modest; Stryker & Microvention. G. Consultant/Advisory Board; Significant; Medtronic.

Funding: No

Funding Component: P272

**Involvement of Neuro-Inflammation in the Pathogenesis of MCT-Induced Pulmonary Hypertension**

**Ravindra K Sharma,** Vinayak Shenoy, Ashok Kumar, Avinash Mandloi, Michael J Katovich, Mohan K Raizada, Univeristy of Florida, Gainesville, FL

**Background:** Pulmonary hypertension (PH) is a devastating disease characterized by increase in pulmonary pressure that eventually leads to right heart failure and death. PH is associated with heightened circulatory cytokines and infiltration of inflammatory cells within the diseased lungs. However, involvement of inflammation within the central nervous system (CNS) in PH pathophysiology has never been investigated. Emerging evidence suggest that activated microglial cells and neuro-inflammation within the CNS play an important role in the pathology of several CNS disorders, including resistant hypertension.

**Objective:** These observations led us to propose the hypothesis that microglial activation and neuro-inflammation in the autonomic brain regions play regulatory role in PH. Minocycline (Mino), an anti-inflammatory antibiotic, which has been reported to inhibit microglial activation in the autonomic brain regions, was used to test this hypothesis.

**Methods:** PH was induced in adult male rats by a single injection of monocrotaline (MCT; 50mg/kg sc). A subset of MCT-injected animals was infused intracerebroventricularly (ICV) with Mino (20mg/ml). After 4 weeks of treatment, animals were sacrificed for the measurement of physiological and pathological parameters.

**Results:** ICV infusion of Mino significantly attenuated right ventricular systolic pressure (RVSP; Con: 30.1±5, MCT: 76.1±14, MCT+Mino: 50.1±11 mmHg) and right ventricular hypertrophy (RVH; Con: 0.26±0.02, MCT: 0.49±0.12, MCT+Mino: 0.38±0.1) induced by MCT. MCT administration resulted in ~2 fold increase in microglial cells, predominantly in the hypothalamic paraventricular nucleus (PVN), an effect significantly attenuated by ICV Mino (Con: 4.0±1.0, MCT: 8.6±1.1, MCT+Mino: 5.0±1.0). MCT injection increased pro-inflammatory cytokines [IL-1β (155%), TNF-α (165%) and IL-6 (113%)] and decreased IL-10 (46%) levels in the PVN. However, ICV Mino treatment restored these cytokines to control levels.

**Conclusion:** Our observations demonstrate that microglial activation in the PVN is involved in PH pathophysiology. They, for the first time, suggest the involvement of neuro-inflammation and autonomic dysregulation in the development and establishment of PH.
Increased Inhibition of Potassium Channel Currents by Angiotensin II in Sympathetic Neurons may Contribute to a Sustained Blood Pressure Elevation in \( \text{(mRen2)27} \) Rats.

Serguei S Sidach, Victor M Pulgar, Azeez A Aileru, Winston Salem State Univ, Winston Salem, NC

It is well established that the increased sympathetic tone may contribute to initiation and progression of various forms of hypertension. Several lines of evidence suggest a link between the renin-angiotensin system and sympathetic nerve activity in hypertension, and the previous studies in animal models demonstrated increased sympathetic output in the presence of Angiotensin II (AngII). To elucidate potential underlying molecular mechanisms of such phenomenon, we compared the effect of AngII on the whole-cell potassium channel currents in superior cervical ganglia (SCG) neurons isolated from hypertensive \( \text{(mRen2)27} \) rats with overexpression of renin gene, and control Sprague Dawley\(^\circ\) (SD) rats. In both groups, the whole-cell potassium channel currents were identified as rapidly-activating, 4-Aminopyridine-sensitive transient A-type currents, as well as slowly-activating tetraethylammonium-sensitive delayed rectifier currents. When the cell membrane was depolarized to -40, -30 and -20 mV from a holding potential of -80 mV, AngII (100 nM) profoundly inhibited A-type current, but the magnitude of such inhibition was not significantly different between neurons isolated from \( \text{(mRen2)27} \) (38.1\(\pm\)6.2\%, 47.8\(\pm\)5.7\% and 52.1\(\pm\)5.7\%; \(n=11\)) and SD rats (37.2\(\pm\)4.6\%, 44.\(\pm\)4.5\% and 46.1\(\pm\)4.8\%; \(n = 13\)). Delayed rectifier potassium channel currents were isolated by holding cells at -40 mV, which resulted in complete elimination of the transient A-type current. In contrast to transient current, inhibition of the delayed rectifier current by AngII in the range of membrane potentials between +20 and +80 mV was significantly greater (\(p<0.05\)) in neurons obtained from \( \text{(mRen2)27} \) rats (11.0\(\pm\)3.2\% to 25.0\(\pm\)2.9\%, \(n=12\)) when compared to SD rats (4.7\(\pm\)1.5\% to 16.3\(\pm\)2.7\%, \(n = 12\)). In both groups, inhibition of both channel types was completely abolished by 10 uM Losartan, indicating involvement of AT1 receptors. Our results suggest that in \( \text{(mRen2)27} \) hypertensive rats, the increased inhibitory effect of AngII on delayed rectifier potassium channel currents could possibly lead to lowering spike threshold, which, in turn, could elevate sympathetic outflow and lead to sustained blood pressure elevation.

Funding: No

Funding Component: P274

Fruit Extract (blueberry, Cranberry, And Promegranate) Improved Insulin Resistance In Overweight Hypertensive And Normotensive Subjects

Ludmila N Novaes, Mariele Moraes, Keyla Katayama, Carine Sangaleti, Maria Claudia Irigoyen, Luiz Bortolotto, Heno Lopes, Heart Inst (InCor) do Hosp das Clinicas da Faculdade de Medicina da USP, Sao Paulo, Brazil

Arterial hypertension is frequently associated to glucose and lipid metabolism abnormalities. The purpose of this study was to determine if antioxidants (fruit extract) supplementation...
interfere with glucose and lipid metabolism in overweight hypertensive patients. A randomized clinical trial was conducted with 30 individuals, 23 hypertensive patients (group A) and 7 normotensive controls (group B). They were randomized to take 3 capsules of different fruits extract a day (blueberry, cranberry and pomegranate) or placebo for 4 weeks. This is a crossover study, which started with placebo changed to capsules and vice versa. Blood samples were collected after 12 hours fasting for biochemical tests (glucose, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), anthropometric assessment (weight, height, and body mass index), systolic BP, diastolic BP and heart rate were evaluated at baseline, after 4, and 8 weeks. The comparisons between groups were held with the GLM repeated measures. Twenty three hypertensive patients (age 47 years, 14 females) and 7 normotensive controls (age 40 years, 7 females) were evaluated. BMI, blood pressure, heart and lipid profile did not differ between groups. HOMAIR decreased significantly in both groups. See results in table 1.

Values are expressed as medians (±SD)
In these preliminary results a 4-weeks supplementation of antioxidants (fruit extract) improved insulin resistance in overweight hypertensive and normotensive subjects. Financial support: FAPESP 2014/25808-3

| Funding: No |
| Funding Component: P275 |

**A Randomized Clinical Trial on Dietary Nitrate and Blood Pressure**

**Michaela L Sundqvist**, Eddie Weitzberg, Jon O Lundberg, Physiology and Pharmacology, Stockholm, Sweden

**Background**
Elevated blood pressure (BP) is a major risk factor for cardiovascular disease and premature death worldwide. Hypertension (i.e. BP of ≥140/90 mmHg) is even considered the leading risk factor for global burden of disease. Dietary strategies preventing hypertension includes the Dietary Approaches to Stop Hypertension eating plan. A vegetarian diet and high vegetable intake has also shown beneficial effects on BP regulation.
In the last 10 years there is an emerging interest in the positive effects of dietary nitrate (NO3−). Inorganic nitrate is converted to nitric oxide (NO) in our body with vasodilatory effects.
Vegetables alone contribute with 60-80% (60-80 of 100) of the total nitrate intake in humans and the highest concentrations of this anion are found in green leafy vegetables and beetroots. Several short term studies have shown that administration of nitrate salts or high nitrate vegetables (HNV) have BP lowering effects. A large scale randomized clinical trial is needed to explore long term effects on BP after daily intake of HNV.

Methods
The Dietary Nitric Oxide (DINO) study is a single site, randomized clinical trial including subjects (n = 300) between 50-70y with SBP 130-159 mm Hg. A wash out period of 2 weeks is followed by randomization to one of three intervention groups: HNV containing 300 mg nitrate + placebo pill, low nitrate vegetables (LNV) + nitrate supplement (300mg potassium nitrate) or LNV + a placebo pill. All subjects will have a constant vegetable intake during 5 weeks of HNV or LNV provided weekly by the investigators, to consume together with their normal diet. After the wash out period and in the last week of the study 24h ambulatory BP, 24h urine collection, and blood- and saliva samples are taken. Lifestyle habits are also monitored during the study. The primary endpoint is systolic ambulatory BP with 85% (85 of 100) power to detect a difference of 2mm Hg between the groups.

Conclusion
The DINO study is designed to investigate the potential contribution of inorganic nitrate to the BP lowering effect attributed to vegetables. In addition, the three armed design makes it possible to evaluate natural sources of nitrate compared to supplements.

M.L. Sundqvist: None. E. Weitzberg: F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Significant; Co-inventor of patent applications relating to the Medical use of nitrate- and nitrite salts. J.O. Lundberg: F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Significant; Co-inventor of patent applications relating to the Medical use of nitrate- and nitrite salts.

Funding: No

Funding Component: P276

Tissue Primacy of Chymase as an Angiotensin II-forming Enzyme from Angiotensin-(1-12)

Sarfaraz Ahmad, Jasmina Varagic, Che Ping Cheng, Wake Forest Sch of Med, Winston-Salem, NC; James F Collawn, Louis J Dell’Italia, Univ of Alabama at Birmingham, Birmingham, AL; Carlos M Ferrario, Wake Forest Sch of Med, Winston-Salem, NC

Breaking the prevailing acceptance of ACE primacy as the Ang II-forming enzyme, we have demonstrated that cardiac Ang II production in human and rat heart tissues are primarily mediated by chymase. In this study, we compared the affinity of cardiac chymase to generate Ang II from Ang-(1-12) or Ang I in plasma membranes (PMs) isolated from the diseased left atria of humans and SHR left ventricle. PMs (50-100 µg) were exposed to increasing concentrations of either Ang-(1-12) or Ang I substrate (0-300 µM) for 30 min at 37°C in the presence of lisinopril (200 µM). The Km and Vmax of human cardiac chymase (Mean ± SE) were 29 ± 0.9 vs 87 ± 8.8 µM and 57 ± 1.4 vs 145 ± 3.7 µM/min/mg for Ang-(1-12) and Ang I substrates, respectively. Similarly, the Km and Vmax of rat cardiac chymase were 64 ± 6.3 vs 142 ± 17 µM and 13.2 ± 1.3 vs 1.9 ± 0.2 µM/min/mg for Ang-(1-12) and Ang I substrate, respectively.
These data suggest that cardiac chymase has a higher affinity for Ang-(1-12) substrate compared to Ang I in both human and rat heart tissues. Further, our kinetic data show that the catalytic efficiency (ratio of $V_{\text{max}}/K_{\text{m}}$) of human and rat chymase were 1.2 and 15.4-fold higher for Ang-(1-12) substrate compared to Ang I. Overall, our findings suggest that Ang-(1-12), rather than Ang I, is the preferred substrate for chymase in the generation of Ang II by human and rat heart tissue.

S. Ahmad: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL-051952.  
J. Varagic: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL-051952.  
C.P. Cheng: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL-051952.  
J.F. Collawn: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL-051952.  
L.J. Dell'Italia: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL-051952.  
C.M. Ferrario: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL-051952.

Funding: No
Funding Component: P277

Altered Cardiomyocyte Inotropic Response to Chymase/Ang-(1-12) Pathway Activation in Heart Failure

Tiankai Li, Xiaowei Zhang, Sarfaraz Ahmad, Jasmina Varagic, Heng-Jie Cheng, Zhi Zhang, Leanne Groban, Carlos M. Ferrario, Che-Ping Cheng, Wake Forest Univ Sch of Med, Winston-Salem, NC

Background. Angiotensin-(1-12) [Ang-(1-12)], a new member of the renin-angiotensin system (RAS) has been identified as a chymase-dependent source for Angiotensin II (Ang II) inotropic activity. Recent observations suggest that activation of this non-canonical chymase/Ang-(1-12) pathway may modulate cardiac function bypassing the inhibitory effects of RAS blockade with ACEI and ARBs in heart failure (HF). However, the direct cardiac effects of Ang-(1-12) and Ang II are unclear. Moreover, whether and how HF alters Ang-(1-12) and Ang II cardiac responses are undefined. We assessed the hypothesis that HF is associated with a reduced action of Ang-(1-12) on myocyte contractility and $[\text{Ca}^{2+}]_i$ regulation.

Methods. We compared LV myocyte contractile and calcium transient ([Ca$^{2+}]_i$) responses to angiotensin peptides in 12 SD rats with isoproterenol induced HF (2 months after isoproterenol 170 mg/kg sq for 2 days) and 16 age-matched controls.

Results. In normal myocytes, versus baseline, Ang II (10$^{-6}$ M) superfusion significantly increased myocyte contraction ($dL/dt_{\text{max}}$) (40%, 184.6 vs 132.1 μm/s), relaxation ($dR/dt_{\text{max}}$) (34%, 141.3 vs 107.0 μm/s) and $[\text{Ca}^{2+}]_i$ (29%,...
Superfusion of Ang-(1-12) (4x10^{-6} M) caused similar increases in dL/dt_{max} (34%) dR/dt_{max} (25%) and [Ca^{2+}]_{iT} (25%). Compared with the changes in normal myocytes, in HF myocytes, Ang II and Ang-(1-12) caused similar but significantly attenuated positive inotropic actions with about 42% to 50% less increases in dL/dt_{max}, dR/dt_{max} and [Ca^{2+}]_{iT}. The Ang-(1-12)-mediated effects were abolished by prior exposure of myocytes to chymostatin in both normal and HF. The Ang II-induced inotropic effects were completely prevented in the presence of an inhibitory cAMP analog, Rp-cAMPS (10^{-4}M 2 h) in both normal and HF myocytes, but were further augmented only in HF after the incubation of myocytes with the G_{i} inhibitor, pertussis toxin (PTX, 2 μg/ml, 36°C, 5h).

Conclusions. Ang-(1-12) stimulates LV myocyte contractile function and [Ca^{2+}]_{iT} in both normal and HF rats through a chymase mediated action. Altered inotropic responses to Ang-(1-12) and Ang II in HF myocytes is mediated through a cAMP-dependent mechanism that is coupled to both stimulatory G and inhibitory PTX-sensitive G proteins.

T. Li: None. X. Zhang: None. S. Ahmad: None. J. Varagic: None. H. Cheng: None. Z. Zhang: None. L. Groban: None. C.M. Ferrario: None. C. Cheng: None.

Funding: No

Funding Component: P278

On the Origin of Brain Renin

Bibi S van Thiel, Luuk te Riet, David Severs, Estrellita Uijl, Ingrid M Garrelts, Erasmus MC, Rotterdam, Netherlands; Fatimunnisa Qadri, Max Delbrück Ctr, Berlin, Germany; Natalia Alenina, Max Delbrück Ctr, Berlin, French Guiana; Michael Bader, Max Delbrück Ctr, Berlin, Germany; A Danser, Erasmus MC, Rotterdam, Netherlands

A renin-angiotensin system (RAS) in the brain is believed to contribute to blood pressure regulation. Given the presence of the blood-brain barrier, brain RAS activity most likely depends on synthesis of (pro)renin in the brain. In support of this concept, an intracellular, non-secreted form of renin has been described in the brain, depending on an alternative transcript of the renin gene. To what degree this intracellular truncated prorenin truly displays angiotensin (Ang) I-generating activity (AGA) is unknown. In the present study, we set out to quantify brain (pro)renin, both before and after buffer perfusion of the brain, in normal mice, renin knockout (KO) mice, DOCA-salt-treated mice (which have been reported to display brain RAS activation), and angiotensin II-infused mice. Brain nuclei were homogenized and incubated with excess angiotensinogen to detect AGA, both before and after acid activation of prorenin, with or without the renin inhibitor aliskiren to correct for non-renin-mediated AGA. Renin-dependent (i.e., aliskiren-inhibitable) AGA was readily detectable in brain nuclei, the highest AGA being present in brainstem (>thalamus =cerebellum =striatum >midbrain >hippocampus =cortex). Brain AGA increased non-significantly after prorenin activation, suggesting that brain prorenin levels are low or absent. Buffer perfusion reduced AGA in all brain areas by >60%. Plasma renin (expressed per mL plasma) was 40-800 x higher than brain renin (expressed per g tissue). Plasma prorenin levels resembled plasma renin levels. AGA was undetectable in plasma and brain of renin KO mice, both before and after prorenin activation. DOCA-salt and Ang II suppressed plasma renin, and parallel decreases were observed for brain renin. In conclusion, brain renin levels (per g tissue) correspond with the amount of renin present in 1-20 μL blood plasma. Brain renin disappears after buffer perfusion, and varies in association with plasma renin. This indicates that renin detected in brain...
Human Angiotensin Receptor Gene Variations, Hypertension & Reno-Vascular Complications

Sudhir Jain, Natalie Sirianni, Nitin Puri, Ahmed Al Khudhair, Ashok Kumar, Univ of Toledo, Toledo, OH

Angiotensin receptor type 1 (AT1R), a G-protein coupled receptor mediates the effect of angiotensin-II and contributes to the pathophysiological consequences of renal-vascular system. AT1R-signaling promotes renal sodium retention, vascular remodeling, hypertension, and end organ damage. Genetic variations that increase AT1R can cause pathological outcomes associated with renin angiotensin system overactivity. However, genetically variable, transcriptional regulation of the human AT1R gene is poorly understood.

In this regard, the human AT1R gene has a haplotype block of four SNPs: T/A at -810, T/G at -713, A/C at -214, and A/G at -153 in its promoter. Variants -810T, -713T, -214A, and -153A always occur together (named haplotype-I or Hap-I) and variants -810A, -713G, -214C, and -153G always occur together (haplotype-II or Hap-II). We have found that hap-I is associated with hypertension in Caucasians. Thus, we generated transgenic (TG) mice with hap-II and I of the hAT1R gene to study its transcriptional regulation in vivo. TG mice with hap-I have higher baseline expression of hAT1R (3.9 folds) in the kidney with increased blood pressure (Hap-I, 126±3 vs. Hap II, 115±4). Since, diet-induced obesity is accompanied by systemic inflammation and redox imbalance that, in turn, alter the cellular transcriptional milieu, we gave Western diet (WD) treatment to our TG mice. Preliminary studies show that transcription factors like USF1, GR and STAT3 binds strongly (2.1; 2.3; 1.7 folds respectively Vs Hap-II) to increase hAT1R expression (5.8 folds) and resulting blood pressure (136±2 vs. 120±3 in Hap II) in TG-mice with hap-I, as compared to hap-II. Complementary experiments show increased inflammatory and redox markers in renal tissues of Hap-I mice, when compared to Hap-II, after WD; including, IL6 (5.9 fold), NOX1 (5.2 fold), CRP (9.8 fold), and TNFα (6.3 fold).

Also, histochemical analysis of kidneys show an elevated pathology in Hap-I TG mice. Thus, haplotype-dependent transcriptional regulation of the hAT1R gene causes increased hAT1R expression and blood pressure, in Hap-I TG mice. Importantly, WD exacerbates this differential gene-expression regulation, further increasing hAT1R and promoting a pro-oxidant/inflammatory milieu in mice with Hap-I.


Funding: No

Novel Insights into Transcriptional Regulation of the Human Angiotensinogen Gene by High-salt: A Study in Transgenic Mice

Meenakshi Kaw, Sudhir Jain, Shravan Perla Perla, Natalie Sirianni, Mariam Alakrawi Alakrawi, Nitin Puri, Sai Sushani Yanamandra, Ashok Kumar, Univ of Toledo, Toledo, OH

Angiotensinogen is the substrate for the entire RAS cascade and polymorphisms leading to its overexpression are linked to hypertension. SNPs
in the promoter of the hAGT gene are associated with hypertension. Importantly, these SNPs can further modulate the gene of interest in various physiological/environmental settings like the high-sodium diet. In this regard, the human angiotensinogen (hAGT) gene has polymorphisms in its 2.5Kb promoter that form two haplotype (Hap) blocks: -6A/G (-1670A/G, -1562C/T, -1561T/C) and -217A/G (-532T/C, -793A/G, -1074T/C, and -1178G/A). Hap -6A/-217A is associated with human hypertension whereas Hap -6G/-217G reduces cardiovascular risk. We have engineered transgenic (TG) mice with these haplotypes (Hap -6A: -6A/-217A and Hap -6G: -6G/-217G) so as to examine the transcriptional regulation of the hAGT in an in vivo setting. This study is designed to study the effects of a high-sodium diet on the transcriptional milieu of renal tissues with consequential effects on the hAGT expression in our two haplotypes. Male TG mice were placed on 4% Na+ diet for a period of 8 weeks. High-salt diet induces mineralocorticoid receptor (MR) and SGK-kinase expression in both haplotypes, equally. MR has been shown to bind to GRE elements in the hAGT gene. Importantly, MR-binding (ChIP assay) and hAGT induction are significantly (p<0.05) greater in the -6A haplotype males as compared to -6G males. High-salt also increased the expression of transcriptional regulators including CEBPβ and HNF4 (p<0.05) that are independent of haplotype. Complementary ChIP assay confirmed enhanced transcription factor (TF) binding to the chromatin of male -6A TG mice as compared to -6G counterparts after high-salt diet treatment. Thus, we show here an effect of high-salt on cellular transcriptional apparatus that is haplotype-independent. However, increased TF affinity of the chromatin in -6A TG mice leads to higher salt-induced AGT levels in this haplotype than -6G. These observations could partly account for increased salt-sensitivity of some adult males that, in turn, is governed by the “risk” haplotype. Identifying these individuals with the -6A haplotype will help guide therapeutic lifestyle changes in patients with essential hypertension.


Funding: No

Funding Component: P281

**Alamandine-induced Vasorelaxation is Selectively Increased in Sp-shr**

**Nadia Leao, Gisele Etelvino, Robson Santos, UFMG, Belo Horizonte, Brazil**

The renin angiotensin system is implicated in hypertension and cardiovascular diseases. It’s actions are dependent on counter-regulatory modulation of its vasopressor and vasodepressor axes. Recently, our laboratory described and characterized a new component the RAS, alamandine and its receptor, the MrgD. Alamandine can be formed from angiotensin A through the action ACE2 or from angiotensin-(1-7), by a still unknown decarboxylase. Among the actions of alamandine, an endothelium-dependent vasorelaxation in aortic rings of mice and rats has been described. The aim of this study was to investigate the vasorelaxing effect of alamandine in aortic rings of hypertensive rats. The vasorelaxing effect of alamandine was tested in aortic rings taken from SP-SHR, Wistar and SHR, pre-contracted with phenylephrine (0.1 µM) with and without enthotelium or in the presence of L-Name (100 µM ) or Indomethacin ( 10 µM). In aortic rings from SP-SHR alamandine produced a pronounced dose-dependent relaxation when compared with Wistar (Emax= 80± 6,0 vs 45 ± 4). This response was diminished in the presence of L-Name (Emax= 39 ± 7 in SP-SHR and 1,0 ± 3 in Wistar). Indomethacin also attenuated the
vasorelaxation produced by alamandine in aortic rings from SP-SHR (Emax = 40 ± 4.0), while a smaller effect was observed in Wistar rats (Emax = 31 ± 4). The vasorelaxing effect of alamandine in SP-SHR was abolished in endothelium desnude rings (Emax= -9 ± 4). When we compare two models of animals with hypertension, SHR-SP and SHR the vasorelaxing effect of alamandine was also more pronounced in SP-SHR (Emax = 80± 6.0 and 38 ± 4 respectively). Taken together these results suggest that the vasorelaxing effect of alamandine is selectively increased in SP-SHR. The mechanism of its effect in SP-SHR appears to involve NO and prostaglandins release.

N. Leao: None. G. Etelvino: None. R. Santos: None.

Funding: No

Funding Component: P282

Use of a CRISPR/Cas9 System for Specification of the Renin Cell Phenotype

Silvia Medrano, Evan Brown, Maria F Martinez, Maria Luisa S Sequeira-Lopez, R Ariel Gomez, Univ of Virginia, Charlottesville, VA

Renin is a key enzyme/hormone that controls blood pressure and fluid-electrolyte homeostasis. Renin transcription is subject to complex developmental, physiological, and pathological regulation. We have identified the cAMP pathway and associated epigenetic marks as crucial regulators of renin expression. However, those studies involved the use of cAMP activators or analogues or histone deacetylase inhibitors that alter the transcriptome and epigenome of the whole cell without exclusively targeting the renin locus. The CRISPR/Cas9 Type II bacterial immune system has been modified for genome editing in eukaryotes. This system consists of a CRISPR-associated endonuclease (Cas9) and single guide RNA (sgRNA) to target specific genomic areas. Modifications to the Cas9 enzyme have allowed the use of CRISPR to regulate gene expression by targeted modification of epigenetic marks.

In this study we tested whether renin expression can be induced by a nuclease-null dCas9 protein fused to the catalytic core of the acetyltransferase p300 (dCas9p300) and sgRNAs directed to the renin promoter and enhancer. We used cultured arteriolar smooth muscle cells of the renin lineage that constitutively express CFP (a renin lineage marker) and YFP only when the renin gene is turned on. Cells were transfected with a dCas9p300 and sgRNA expression vectors and analyzed for YFP expression 48h after transfection. We tested four sgRNAs targeting the renin enhancer and five sgRNAs targeting the renin promoter either individually or in combination. We found few YFP+ cells when all 4 enhancer sgRNAs or 5 promoter sgRNAs were simultaneously used. The highest YFP expression (32±9 cells/well) was observed when two enhancer sgRNAs (at positions -2,757 and -2,631) and three promoter sgRNAs (at positions -719, -50 and -25) were simultaneously added to the cells. We did not find any YFP+ cells when the dCas9p300 plasmid was transfected without sgRNAs or when cells were transfected with a control plasmid.

Our data support targeted acetylation as a causal mechanism of renin transactivation. CRISPR/Cas9 provides a tool to study the regulation of renin expression by targeting epigenetic marks in the promoter and enhancer of renin.

S. Medrano: None. E. Brown: None. M.F. Martinez: None. M.S. Sequeira-Lopez: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH NDDK. R.A. Gomez: B. Research Grant (includes principal investigator, collaborator, or...
consultant and pending grants as well as grants already received); Significant; NIH NIDDK NHLBI.

Funding: No
Funding Component: P283

**Effects of Ovarian Estrogens on Local RAS Activity in Cardiomyocytes and Non-cardiomyocytes**


The relatively low efficacy of ACE-Is in the treatment of heart failure (HF) in women after estrogen (E2) loss may be due to their inability to reach the intracellular sites at which Ang II is generated and/or the existence of cell-specific mechanisms in which ACE is not the essential processing pathway for Ang II formation. We compared the metabolic pathway for Ang II formation in freshly isolated cardiomyocytes (CMs) and non-cardiomyocytes (NCMs) extracted from hearts of gonadal-intact and ovariectomized (OVX) adult WKY and SHR rats. Circulating levels of angiotensinogen (AOGEN) were higher in WKY (958 ± 47 pg/mL) vs. SHR (SHR: 626 ± 40 pg/mL; P<0.05 strain effect) and E2 loss augmented this effect in WKY (WKY OVX: 1,169 ± 66 pg/mL vs. SHR OVX: 625 ± 41 pg/mL). Correspondingly, plasma Ang II levels were higher in WKY vs. SHR (strain effect: WKY: 62 ± 6 pg/mL vs. SHR: 42 ± 9 pg/mL), independent of OVX. Chymase activity was nearly 40-fold higher in NCMs compared to CMs, and for the NCMs, activities were highest in cells from WKY vs. SHR and OVX vs. intact rats (P<0.05 strain and E2 effects, respectively) (Figure).

Neither strain nor gonad status influenced the lower ACE activity found in both NCMs and CMs. In contrast, ACE2 activity in CMs and NCMs was higher in cells from WKY vs. SHR (P<0.05 strain effect), independent of E2 status. We conclude that NCMs from WKY and SHR express significantly higher levels of chymase, ACE, and ACE2 activities. E2 loss leads to selective changes in the activity of chymase, but not ACE, in NCMs. The significance of these novel findings is that targeted cell-specific chymase rather than ACE inhibition may have a greater benefit in the management of HF in women after menopause.

**Figure**

X. Sun: None. S. Ahmad: None. M. Lin: None. G. Zapata-Sudo: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Instituto Nacional de Ciencia e Tecnología de Farmacos e Medicamentos (INCT-INOFAR). C. Cheng: None. J. Varagic: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; HL-051952. C. Ferrario: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants...
Angiotensin II Upregulates Cytochrome-450 4A Expression in Rat Kidney Through Type 1 Receptor

Wanting Wang, Rong Rong, Osamu Ito, Yoshiko Ogawa, Yoshikazu Muroya, Masahiro Kohzuki, Tohoku Univ, Sendai, Japan

20-hydroxyeicosatetraenoic acids (20-HETE) is a cytochrome P-450 (CYP) 4A-dependent metabolite of arachidonic acid and regulates vascular tone and renal tubular function. Previous studies showed that angiotensin II (Ang II) stimulated the renal CYP activity and 20-HETE production through the Ang II type 1 (AT1) receptor and that the Ang II-increased the 20-HETE was linked to the Ang II type 2 (AT2) receptor. Thus, the study was designed to clarify the role of Ang II in CYP4A isoforms expression in the rat kidney. Male Sprague-Dawley rats were infused Ang II at low dose (AL, 0.17mg/kg/min, sc) and high dose (AH, 0.70mg/kg/day, sc) by using osmotic mini pump, with or without AT1 receptor blocker candesartan (1 and 3mg/kg/day, po) for 1 week. The protein expression of CYP4A isoforms, AT1 receptor and AT2 receptor in the renal cortex, outer medulla, and inner medulla was examined by immunoblot analysis. The mRNA expression of CYP4A isoforms was examined by reverse transcription and polymerase chain reaction (RT-PCR). Ang II at high dose increased systolic blood pressure (control, 109±2; AH, 164±8 mmHg, p<0.01), creatinine (control, 0.24±0.0; AH, 0.29±0.01 mg/dl, p<0.01) and urinary albumin excretion (control, 20.3±5.9; AH, 2398.6±303.6 μg/mg creatinine, p<0.01). In the control group, the CYP4A1, 4A2, and 4A8 proteins were highly expressed in the renal cortex, lowly expressed in the outer medulla, barely detected in the inner medulla. The AT1 receptor was expressed in kidney sections; highly in the outer and inner medulla, the AT2 receptor was only detected in the outer medulla. Ang II dose-dependently increased all CYP4A isoform proteins in the renal cortex and outer medulla (CYP4A1, 24% and 222%; CYP4A2, by 51% and 258%; CYP4A8, by 52% and 550%, p<0.05). Ang II also increased all CYP4A isoform mRNAs in the renal cortex and outer medulla. The candesartan treatment dose-dependently inhibited the Ang II-increased blood pressure, creatinine, urinary albumin excretion and CYP4A isoform expressions. These results indicated that Ang II increases CYP4A isoform expressions in the kidney through AT1 receptor. The Ang II-upregulated CYP4A expressions may play an important role in hypertension and renal function.

W. Wang: None. R. Rong: None. O. Ito: None. Y. Ogawa: None. Y. Muroya: None. M. Kohzuki: None.

Diastolic Dysfunction After Estrogen Loss is Linked to Cardiac Chymase in WKY but Not SHR Rats

Hao Wang, Wake Forest Sch of Med, Winston Salem, NC; Jacqueline da Silva, Daniele Gabriel-Costa, Univ Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Sarfaraz Ahmad, Wake Forest Sch of Med, Winston-Salem, NC; Xuming Sun,
Left ventricular diastolic dysfunction (LVDD) develops in response to hypertension and estrogen (E2) loss and is consequent to heart failure in women. To understand the mechanisms underlying the development of LVDD as a result of the interaction between E2 loss and the cardiac RAS, we compared the relationships of LV tissue RAS components and E/e′ between adult SHR (n=13) and WKY (n=9) female rats after ovariectomy (OVX) or sham surgery (intact). In intact rats, E/e′ was higher in SHR vs. WKY rats (P<0.05 strain effect) and after OVX, the diastolic phenotype of WKY’s mimicked that of intact SHR counterparts (Figure).

While relationships between RAS enzymatic activities and E/e′ were not significant in SHRs with respect to estrogen status (data not shown), OVX-induced increases in E/e′ were significantly linked to increases in chymase gene expression and enzymatic activity in the WKY strain (Figure). These data indicate that 1) the altered diastolic function in SHR is relatively insensitive to loss of estrogen while the opposite is true in WKY rats, and 2) OVX-induced LVDD in WKY is directly related to increases in cardiac chymase activity. Further elucidation of the interplay between an activated cardiac chymase-mediated RAS metabolism and LVDD following estrogen loss in normotensive subjects is warranted.
consultant and pending grants as well as grants already received); Significant; Instituto Nacional de Ciencia e Tecnologia de Farmacos e Medicamentos (INCT-INOFAR). L. Groban: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL-051952; AG033727.

Funding: No
Funding Component: P286

Modulation Way Classical and Alternatives of Renin Angiotensin System (RAS) in Mesangial Cells After Exposure to Fructose

Rodrigo Yokota, Zaira Jara Palomino, Larissa Emi Matsumoto, Danielle Sanches Aragão, Dulce Elena Casarini, Nephrology Discipline, Med Dept, Federal Univ of Sao Paulo, Sao Paulo, Brazil

Background: Our group demonstrated previously that rats subjected to a high fructose diet showed increased activity of kidney ACE. In order to understand the effect of fructose in the forming process of angiotensin II (AngII) and angiotensin 1-7 (Ang1-7) affecting the levels of these effectors, the aim of this study was to evaluate the activities of the RAS enzymes responsible for the formation of Ang1-7 and AngII of immortalized human mesangial cells (IMCH) exposed to low and high concentration of fructose.

Methods: IMCH were divided into control (low glucose medium), Fructose 5 mM (medium + 5 mM Fructose) and Fructose 30 mM (medium + 30 mM Fructose) groups. The enzymatic activity of ACE, chymase, cathepsin D, Neutral Endopeptidase (NEP) and ACE2 were evaluated using Z-Phe-His-Leu, Abz-AIKFFSAQ-EDDnp, Abz-AIAFFSRQ-EDDnp, Abz(d)-Arg-Gly-Leu, Mca-APK (DNP) as substrates respectively. Data were analyzed using ANOVA one way.

Results: Among the AngII forming enzymes, fructose decreased ACE activity in cell lysate. Both fructose concentrations increased chymase in the culture medium. No effect was observed on the activity of cathepsin D. When evaluating the activities of peptidases forming Ang1-7, NEP activity was increased in culture medium. We did not detect differences in ACE2 activity.

Conclusion: The results suggest fructose modulates renal and systemic RAS in IMCH acting in the classical and alternative pathways of AngII formation. Additionally, this sugar was able to increase NEP activity, forming Ang1-7. Quantitation of angiotensin remains necessary to better understand how the end effector is available in IMCH and evaluate possible effects on glomerular physiology.


Funding: No
Funding Component: P287

Endothelial Dysfunction in Small Resistance Arteries of Patients with Severe Obesity: Role of Arginase

Agostino Virdis, Emiliano Duranti, Monica Nannipieri, Marco Anselmino, Andrea Grazi, Stefano Taddei, Univ of Pisa, Pisa, Italy

Nitric oxide (NO) is produced by endothelial NO synthase (eNOS) using the aminoacid L-Arginine. Arginase (Arg) also uses L-Arginine as substrate,
converting it in L-Ornitine and urea. An increased Arg activity causes a progressive L-Arginine depletion, which in turn determines a lower NO bioavailability. Studies in murine models of obesity identify Arg as a determinant of endothelial dysfunction. In this study, we evaluated whether Arg might play a role in determining the lower bioavailability of NO in small resistance arteries isolated from subcutaneous tissue of patients with severe obesity (Ob), split in age groups (younger than 30 aa, range 21-29, n=5; older than 30 aa, range 35-56, n=5) vs normoweight controls (Ctrl younger 30 years, range 20-29, n=5; older than 30 yrs, range 36-58, n=5). Each patient underwent a subcutaneous biopsy during a laparoscopic surgical procedure. Small arteries, isolated from periadvential fat, were evaluated on a pressurized micromyograph. Endothelium-dependent vasodilation (VD) was assessed by acetylcholine (Ach, 0,001-100μM). NO availability was assessed by repeating Ach with L-NAME (100μM). Ach was also infused in the presence of norNOHA (10μM, Arg inhibitor). In Ctrl, VD induced by Ach was inhibited by L-NAME and not modified by norNOHA. Ob younger exhibited a reduced VD induced by Ach vs Ctrl of the same age, a reduced inhibition by L-NAME, and a potentiating effect by norNOHA, which also normalized the inhibitory effect of L-NAME on Ach. In Ob older, VD induced by Ach was reduced vs Ob younger, resistant to L-NAME and not modified by norNOHA.

In conclusions, in small arteries from younger Ob, the Arg inhibition improves endothelial function by increasing the NO availability, while in older Ob Arg does not seem to play any role in endothelial dysfunction.


Funding: No

Funding Component: P288

Action of CMF-019, the First Small Molecule Apelin Receptor Agonist Biased Towards G-protein Signalling versus the β-arrestin/internalization Pathway

Anthony Davenport, Cai Read, Univ of Cambridge, Cambridge, United Kingdom; Christopher Fitzpatrick, Univ of Leeds, Leeds, United Kingdom; Peiran Yang, Rhoda Kuc, Janet Maguire, Robert Glen, Univ of Cambridge, Cambridge, United Kingdom; Richard Foster, Univ of Leeds, Leeds, United Kingdom

Apelin is down-regulated in pulmonary arterial hypertension (PAH) and infusion of apelin is beneficial in animal models. Apelin has a short half-life and is rapidly internalised and inactivated by its receptor through β-arrestin signalling. In PAH, we hypothesise that a G-protein biased small molecule apelin agonist would replace the missing endogenous peptide to produce vasodilatation and protect against cardiac remodelling of the right ventricle without receptor desensitisation. We characterised, in vitro and in vivo, the pharmacology of a novel small molecule agonist, CMF-019, demonstrating G-protein bias at the apelin receptor. CMF-019 bound to human, rat and mouse apelin cardiac receptors with high affinity (pKi= 8.58±0.04, 8.49±0.04 and 8.71±0.06 respectively). In cell-based functional assays, CMF-019 displayed similar potency for the Gαi pathway as the endogenous agonist, [Pyr+]apelin-13 (pD2=10.00±0.13 n=11/4 vs pD2=9.34±0.15 n=8/4 respectively) but was much less potent in β-arrestin (pD2=6.65±0.15 n=13/4 vs pD2=8.65±0.10 n=12/4) and internalisation (pD2=6.16±0.21 n=6/2 vs pD2=9.28±0.10 n=6/2). Results are expressed as mean±sem, n-values are given as the number of replicates/number of experiments. CMF-019 was ~400 biased for signalling through Gαi compared to the β-arrestin pathway and was ~5800 fold biased compared with the receptor.
internalisation assay. Normotensive male Sprague-Dawley rats (273 ± 6g) were induced and maintained under anaesthesia with inhaled isoflurane (3% and 1.5% respectively) carried by oxygen (1.5l/min) and a pressure-volume catheter placed in the left ventricle to measure cardiac parameters. Intravenously injected CMF-019 (2500µg) caused a significant increase in cardiac contractility (dP/dt\(_{\text{Max}}\), 833±152mmHg/s n=9) compared to saline (88.7±94.4mmHg/s n=3) (p<0.001, student’s t-test). CMF-019 is the first biased small molecule identified at the apelin receptor and displays activity in vivo. The results provide evidence that biased agonism can be retained in small drug-like molecules. The study provides a basis for the rational design of new biased apelin receptor agonists for the treatment of cardiovascular conditions such as PAH.

A. Davenport: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Wellcome Trust, British Heart Foundation, Medical Research Council, GSK, Heptares. G. Consultant/Advisory Board; Modest; ViiV.

C. Read: None.

C. Fitzpatrick: None.

P. Yang: None.

R. Kuc: None.

J. Maguire: None.

R. Glen: None.

R. Foster: None.

Funding: No

Funding Component: P289

**Renal Dopamine D2 Receptor Overexpression Ameliorates Ischemia/Reperfusion Injury**


We have shown that long-term renal-selective silencing of Drd2 increases renal expression of proinflammatory and profibrotic factors and blood pressure (BP) in mice. Renal-selective DRD2 rescue by the retrograde renal infusion of adeno-associated virus (AAV) vector with DRD2 (DRD2AAV) reduced the expression of proinflammatory factors and kidney injury, preserved renal function, and normalized systolic and diastolic BP. We hypothesized that increasing DRD2 expression is beneficial not only in conditions associated with reduced DRD2 expression but also in renal damage produced independent of DRD2 reduction. To test this hypothesis, we determined whether DRD2AAV treatment provides protection in a model of renal ischemia/reperfusion (IRI) injury. We studied the effects of 45 min bilateral renal ischemia immediately followed by retrograde renal ureteral infusion of CAAV (control AAV) or DRD2AAV and reperfusion. The mice were studied 14 days later when AAV-mediated DRD2 expression reaches maximum expression. DRD2 expression was increased about 9-fold in the kidneys. The mRNA expressions of TGF-β (1.0±0.02 vs 0.75±0.02; P<0.05; n=3/group), FN1 (1.0±0.03 vs 0.58±0.02; P<0.05, n=3/group), and Col1a1 (1.0±0.01 vs 0.41±0.01; P<0.05, n=3/group) were higher in mice infused with CAAV than in those infused with DRD2AAV. H&E staining of the kidney sections showed that mice treated with CAAV had more renal damage than those treated with DRD2AAV. Systolic BP was higher at the end of the experiment in mice treated with CAAV (baseline: 99±4 vs IRI:116±3 mmHg; P<0.05; n=3/group) than in those treated with DRD2AAV (baseline: 104±4 vs 103±4 mmHg, n=3/group) and were not different from the baseline BP of the CAAV-treated mice. Serum creatinine levels were also higher in mice treated with CAAV than in those treated with DRD2AAV (0.32±0.03 vs 0.21±0.02 mg/dl; P<0.05, n=3/group). Renal susceptibility genes may determine the occurrence and severity of hypertension-induced progressive renal damage but the role of factors that may slow the progression of renal disease is much less studied. Our results suggests that the
development of therapies directed to increase renal DRD2 expression/function may provide novel and effective approaches in the treatment of renal injury.


Funding: No
Funding Component: P290

**Human Stomach Gastrin is Regulated by Sodium Human Stomach Gastrin Secretion is Regulated by Ingested Sodium and the Dopamine 1 Receptor**

**Peng Xu**, John J Gildea, Chi Zhang, Dora Wang, Hahn T Tran, Univ of Virginia, Charlottesville, VA; Pedro A Jose, George Washington Univ Sch of Med, Washington DC, DC; Robin A Felder, Univ of Virginia, Charlottesville, VA

Dietary sodium stimulates the renal excretion of sodium long before there is expansion of the extracellular fluid volume. We have previously shown that an increase in sodium concentration in the incubation buffer in human SW626 colon adenocarcinoma cells that express gastrin-secreting G-cells increased gastrin mRNA and protein. Thus, we hypothesized that increasing extracellular sodium would also increase gastrin expression in normal human stomach G-cells. The highest sodium concentration (170 mM) increased gastrin protein expression significantly (all cell lines were normalized to their gastrin expression at 90 mM, sodium). In normal human G-cells cultured in non-polarized conditions, the normalized gastrin expression in cells incubated in 143 mM sodium was 0.98 ± 0.06; 170 mM sodium increased gastrin expression 1.515 ± 0.106-fold (n=4 different cell lines, P<0.01 one-way ANOVA). Cultured human renal tubule cells demonstrate more physiologically relevant phenotype when grown in a 3D cell culture (3DCC) system. Therefore, we also studied human G-cells grown in 3DCC. Normal human G-cells grown in 3D also had a greater gastrin expression when incubated in 170 than 143 mM sodium: 1.08 ± 0.12 vs. 0.62 ± 0.04, n=4, P<0.05, t-test). We have also shown that the dopamine type 1 receptor (D1R) is expressed in G-cells and may play a role in regulation of gastrin expression. We now report the presence of the complete dopamine biosynthetic pathway in human G-cells: tyrosine hydroxylase is expressed ubiquitously in the stomach while DOPA decarboxylase is expressed selectively in G-cells where D1R is also expressed. We have reported that in the kidney, gastrin, via CCKBR and dopamine, via D1R synergistically increase renal sodium excretion. Thus, the pathway that is involved in the increase in sodium excretion after the ingestion sodium involves sodium- and D1R-mediated increase in gastrin expression in G-cells, followed by secretion of gastrin into the circulation to interact with kidney mediated gastrin pathways.

P. Xu: None. J.J. Gildea: None. C. Zhang: None. D. Wang: None. H.T. Tran: None. P.A. Jose: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5P01HL074940-12. R.A. Felder: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5P01HL074940-12.

Funding: No
Funding Component: P291

**High Fat Diet Promotes Hypertension and Vascular Remodeling but Not Vascular Fibrosis or Altered Contractility in Dahl Salt Sensitive Rats**

**Roxanne Fernandes**, Patricia A Perez Bonilla, Hannah Garver, James J Galligan, Gregory D
Obesity associated hypertension in rodent models is commonly associated with altered vascular reactivity to sympathetic neurotransmitters and inflammation-induced vascular remodeling/fibrosis. Dahl salt-sensitive (SS) rats exhibit elevated sympathetic activity and vascular remodeling. We hypothesized that diet-induced obesity in Dahl SS rats would promote hypertension, vascular dysfunction and remodeling/fibrosis. Male Dahl SS rats were placed on high fat diet (HFD, 60% kcal from fat with final concentrations of 0.33% NaCl and 1% K⁺, n=5) or normal-fat diet (NFD; 10% kcal from fat, 0.24% NaCl, 0.36% K⁺, n=5) for 24-26 weeks after weaning (3 weeks of age). Compared with NFD rats, HFD rats displayed severe hypertension (MAP, 165±4 mmHg vs 133±6 mmHg, P<0.05), higher body-weight (470±6g vs 433±7g, P<0.05), and hyperlipidemia (cholesterol, 211±22 mg/dl vs 138±23 mg/dl, P=0.05). HFD rats did not show significant changes in plasma levels of fasting glucose (85±5 mg/dl vs 75±5 mg/dl, P=0.05), higher body-weight (470±6g vs 433±7g, P<0.05), and hyperlipidemia (cholesterol, 211±22 mg/dl vs 138±23 mg/dl, P=0.05). HFD rats did not show significant changes in plasma levels of fasting glucose (85±5 mg/dl vs 75±5 mg/dl), insulin (2.6±0.8 ng/ml vs 2.2±1.1 ng/ml), leptin (0.77±0.18 ng/ml vs 0.44±0.06 ng/ml), or aldosterone (249±3 pg/ml vs 234±3 pg/ml) (all P>0.05). HFD did not affect pressurized mesenteric arterial (~300 μm inner diameter, 60 mmHg) reactivity to norepinephrine or ATP in vitro. Pressurized mesenteric arteries from HFD rats displayed thicker walls (Ca²⁺ free buffer, 40±1 μm vs 36±1 μm, P<0.05), but showed slightly increased distensibility. Morphological studies did not reveal greater fibrosis in adventitia of mesenteric, intrarenal and coronary arteries from HFD rats. However, HFD induced inflammation in mesenteric perivascular adipose tissue, as shown by increased CD3 positive cell infiltration and histological evidence of fibrosis and angiogenesis. Our studies indicate that HFD in male Dahl SS rats promotes hypertension, perivascular adipose tissue inflammation and vascular remodeling, but not vascular fibrosis. Alteration of vascular contractility to sympathetic neurotransmitters, however, is not required for obesity associated hypertension in Dahl SS rats.

**R. Fernandes:** None.  **P.A. Perez Bonilla:** None.  **H. Garver:** None.  **J.J. Galligan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NHLBI 2P01HL07687.  **G.D. Fink:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NHLBI 2P01HL07687.  **H. Xu:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NHLBI 2P01HL07687.

**Funding:** No

**Funding Component:** P292

**ENPP1-Fc Protein Inhibits Proliferation of Human Vascular Smooth Muscle Cells**

**Yan Yan,** Anumeha Shah, Ashmita Saigal, Susan Faas, Zhiliang Cheng, Kim Askew, Andre Marozsan, Alexion Pharmaceuticals, New Haven, CT

Background: Arterial stenosis leading to hypertension or heart failure is common in patients with Generalized Arterial Calcification of Infancy (GACI), an ultra-rare disease associated with loss of function mutations in ENPP1, an ectonucleotide pyrophosphatase that hydrolyzes extracellular ATP. GACI is characterized by accelerated calcification and severe myointimal proliferation. The role of ENPP1 in myointimal proliferation is unknown. Here, we examined the effect of ENPP1 on proliferation of human vascular smooth muscle.
Methods: ENPP1 expression was assessed in primary hVSMCs using qRT-PCR. ENPP1 expression was silenced using siRNA and its activity was verified by a cell based assay or inorganic pyrophosphate (PPI) assay and its effect on VSMC proliferation was determined by 3H-thymidine incorporation.

Results: Treatment of primary hVSMCs with siRNA specific to the hENPP1 resulted in ~90% decrease in ENPP1 levels. Cellular enzyme activity correlated with ENPP1 expression in VSMCs. Silencing ENPP1 in hVSMCs led to 1-3 fold increase in proliferation relative to that of cells transfected with control siRNA in 2 out of 2 donors. Peak cell proliferation was observed at 5 days post-transfection. Human iPSC derived VSMCs (iVSMCs) expressed higher levels of ENPP1 than primary hVSMCs. Silencing ENPP1 in iVSMCs resulted in 3-5 fold increase in proliferation relative to that of cells transfected with negative control siRNA in 2 out of 2 donors. Addition of recombinant ENPP1-Fc protein restored ENPP1-associated proliferation in all donors (8099.75 ±134.32 (untreated) vs 1478 ± 55.34 (5ug/ml ENPP1-Fc treated, P<0.001)). In contrast, bisphosphonates, a current off-label treatment for patients with GACI, had no effect on cellular proliferation. Increased levels of PPI were also detected in culture supernatants obtained from cells treated with the ENPP1-Fc protein.

Conclusion: For the first time, we have demonstrated that ENPP1 knockdown promotes proliferation of human VSMCs, and treatment with a functionally active ENPP1-Fc protein significantly inhibits ENPP1-associated proliferation. These results suggest that ENPP1 enzyme replacement may be a potential strategy to treat myointimal proliferation in patients with GACI disease.

Y. Yan: A. Employment; Modest; Alexion pharmaceutical. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; ownership of ALXN stock. A. Shah: A. Employment; Modest; Alexion Pharmaceuticals Inc., F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; ownership of ALXN stock. A. Saigal: A. Employment; Modest; Alexion Pharmaceuticals Inc.,. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; ownership of ALXN stock. S. Faas: A. Employment; Modest; Alexion Pharmaceuticals Inc.,. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; ownership of ALXN stock. Z. Cheng: A. Employment; Modest; Alexion Pharmaceuticals Inc.,. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; ownership of ALXN stock. K. Askew: A. Employment; Modest; Alexion Pharmaceuticals Inc.,. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; ownership of ALXN stock. A. Marozsan: A. Employment; Modest; Alexion Pharmaceuticals Inc.,. F.
Endothelial Sodium Channel Activation Promotes Vascular Stiffness in Obese Female Mice


Obesity-associated arterial stiffening is an independent predictor of cardiovascular disease (CVD) events. Although premenopausal non-obese women are protected against CVD, aortic stiffness in obese women is more common than in men. This disproportionate increase in vascular stiffness in obese females may partly explain their loss of sex-related CVD protection. Recent studies have suggested a role for endothelial sodium channel (ENaC) activation in promotion of endothelial stiffness and suppression of flow-(nitric oxide) mediated vasodilation. Increased mineralocorticoid receptor (MR) activation mediated endothelial stiffness is promoted, in part, by ENaC activation. In this regard, we have recently reported increased aortic stiffness, MR and ENaC expression and endothelial dysfunction in female mice fed a high fat and high fructose diet (western diet [WD]). This increase in aortic stiffness was prevented by very low dose MR antagonism. Accordingly, we hypothesized that inhibition of MR-mediated ENaC activation by using a very low dose of the ENaC inhibitor, amiloride, would prevent arterial stiffening and vascular dysfunction in WD-fed female mice. Four week old C57BL6/J mice were fed a WD containing high fat (46%), sucrose (17.5%), and high fructose corn syrup (17.5%) with or without a very low dose of amiloride (1mg/kg/day) for 16 weeks. Amiloride significantly attenuated WD-induced increases in aortic stiffness in vivo as measured by pulse wave velocity as well as in vitro endothelial stiffness as measured by atomic force microscopy. Moreover, incubation of aortic explants with very low dose of amiloride (1 μM) inhibited WD-induced aortic stiffness in aorta explants from WD-fed female mice. Amiloride also prevented WD-induced impairment in acetylcholine-induced aortic vasodilatation and flow-mediated dilation in mesenteric arteries. Taken together, these observations support a role for ENaC activation in diet-induced vascular stiffening in obese females.
Endothelial Sodium Channel Activation Promotes Cardiac Stiffness in Obese Female Mice


Cardiac diastolic dysfunction (DD) and diastolic heart failure is increasing in concert with obesity and aging population in the United States. In obese and diabetic women, DD is more common than in their male counterparts. This disproportionate increase in DD in obese females may partly explain their loss of sex-related cardiovascular (CV) disease protection. Recent studies have suggested a role for endothelial sodium channel (ENaC) activation in promotion of endothelial stiffness and suppression of flow- (nitric oxide) mediated vasodilation. Moreover, increased mineralocorticoid receptor (MR) activation mediated endothelial stiffness is promoted, in part, by ENaC activation. In this regard, we have recently reported increased plasma aldosterone levels, aortic and cardiac stiffness, and cardiac and vascular MR expression in female mice fed a high fat and high fructose diet (western diet [WD]). This increase in CV stiffness was prevented by very low dose MR antagonism. Accordingly, we hypothesized that inhibition of MR-mediated ENaC activation by using a very low dose of the ENaC inhibitor, amiloride would prevent cardiac stiffening (DD) in WD-fed female mice. Four week old C57BL6/J mice were fed a WD containing high fat (46%), sucrose (17.5%), and high fructose corn syrup (17.5%) with or without a very low dose of amiloride (1mg/kg/day) for 16 weeks. Amiloride significantly attenuated WD-induced impairment of cardiac relaxation in vivo as measured by high resolution magnetic resonance imaging (MRI) as well as cardiac interstitial fibrosis as measured by immunohistochemistry by picrosirius red staining. Moreover, amiloride prevented the development of DD in obese female mice without having effects on blood pressure. These observations support a role for ENaC activation in diet-induced cardiac stiffening (DD) in obese females.

J. Habibi: None. A.R. Aroor: None. L. Ma: None. G. Jia: None. A. Whaley-Connell: None. M. Garro: None. B.J. Barron: None. V.G. DeMarco: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Boehringer Ingelheim. J.R. Sowers: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; National Institutes of Health, VA Merit Award.

Funding: No
Funding Component: P296

Effective Arterial Elastance and Hypertensive Response to Stress

Andy Kieu, Michael Widlansky, Medical Coll of Wisconsin, Wauwatosa, WI

Effective arterial elastance (Ea) has shown to be of importance in resting hypertension, though its role is unclear in hypertensive response to exercise or dobutamine. Prior studies have shown that individuals with hypertensive response to exercise (HRE) have diastolic dysfunction as shown on echocardiogram, but there are limited data to show if there are any correlations with Ea. In this retrospective cohort study, we reviewed 415 stress echocardiograms (treadmill and dobutamine) that were performed between 2009 and 2011 that were completed on the
same day as a full resting TTE at our institution. Following exclusion of subjects with coronary artery disease, resting LV ejection fraction less than 50%, resting regional wall motion abnormalities and inducible ischemia, 182 subjects who underwent treadmill stress echocardiogram (TSE) and 149 subjects who underwent dobutamine stress echocardiogram (DSE) were left for analysis. We determined univariate and multivariate predictors of a HTN response to each type of stress test by logistical regression. Covariates included age, sex, resting systolic pressure, resting diastolic pressure, resting hypertension, pulse pressure, Ea, septal e’, septal a’, mitral E/e’, mitral inflow E/A ratio, LV mass index, and left atrial size.

In the TSE group, 26 subjects had hypertensive responses compared to 11 in the DSE group. Resting hypertension was predictive of HRE in both groups. Ea was not predictive of HRE in the TSE group, but was predictive for those in the DSE group (OR 5.91, p=0.03). Increasing resting Ea predicts hypertensive responses to dobutamine, but not exercise. Traditional diastolic Doppler parameters are not significantly associated with hypertensive responses to either stressor.


Funding: No
Funding Component: P297

Distinct Profiles of Extracellular Vesicles are Elevated During Evolution of Hypertension

Uta Erdbruegger, Nancy L Howell, Christine Rudy, Robert M Carey, Thu H Le, Univ of Virginia Health System, Charlottesville, VA

Despite the significant deleterious impact of hypertension (HTN), the blood pressure (BP) threshold at which to treat remains a matter of debate. Early and non-invasive biomarkers of end-organ damage in HTN are needed to optimize treatment for patients with HTN. Circulating microparticles (MPs) are potential candidate biomarkers to reflect early end-organ damage in HTN. MPs are submicron vesicles shed from various cell types in the blood and carry markers from their parent cells. We hypothesize that these MPs show a distinct pattern during evolution of HTN in spontaneous hypertensive rats (SHR), a model of essential HTN. MPs were generated from platelet poor plasma from tail vein of SHR before development of HTN (7 weeks (wk) of age), at time of development (9 wk) and severe HTN (12 wk). WKY rats served as control. Enumeration and phenotyping of MPs was performed with imaging flow cytometry using CD42 (platelet marker), CD31 (PECAM), CD105 (S-endoglin), CD45 (leukocyte) and Annexin V (AnV) as surface markers. Compared to WKY (n=3), all SHR (n=3) had a significant increase in systolic BP by 40mmHG (by radiotelemetry) (p=0.02). All types of MPs increased at wk 9 compared to wk 7 and then decreased again at wk 12 in both groups. However, the increase of MPs was numerically higher in a subgroup of endothelial MPs in SHR after 12 wk compared to controls (AnV negative and S-endoglin (CD105) positive, median 34333 vs 10328 MPs/μl plasma, p=0.08). Subgroups of circulating MPs show a distinct pattern in the SHR. It remains to be determined whether these vesicles reflect early end-organ damage in HTN and have the potential to guide treatment.


Funding: No
Funding Component: P298

Finerenone Protects Against the Acute and Chronic Consequences of Renal Ischemia/reperfusion Injury
Introduction: One of the most common causes of acute kidney injury (AKI) is renal ischemia/reperfusion (IR). Mineralocorticoid receptor (MR) antagonism has shown beneficial effects against renal IR consequences. The potential benefit of novel non-steroidal MR antagonists such as finerenone has not been explored.

Objective: Evaluate the efficacy of finerenone to prevent the acute and chronic consequences of ischemic AKI.

Methods: For the acute study (24 hours), 18 rats were divided in: sham, rats subjected to bilateral renal ischemia of 25 min and rats that received three doses of finerenone at -48 h, -24 h and -1 h before the ischemia. For the chronic study (4 months), 21 rats were divided in: sham, rats with 45 min of bilateral ischemia and rats treated with Finerenone at day -2, -1 and 1h before IR. The left kidney was used for histology and the right kidney for molecular analysis.

Results: After 24 h of reperfusion, the untreated IR rats presented a 3-fold increase in plasma creatinine, accompanied by 40% of tubules presenting cell detachment and casts. Kim-1 and NGAL mRNA levels were induced by 30-fold. In contrast, the rats that received finerenone presented normal creatinine and significantly fewer injured tubules (11%) and a less pronounced induction of kim-1 and NGAL (8-fold). After 4 months, the untreated IR rats developed chronic kidney disease (CKD), evidenced by kidney dysfunction, increased proteinuria (121.6 vs. 14.3 mg/24h in sham) and renal vascular resistance (16.8 vs. 11.4 mmHg/mL in sham). Tubular dilation, extensive tubule-interstitial fibrosis and an increase in kidney TGF-β and Collagen-I mRNA levels also characterized CKD. The transition from AKI to CKD was fully prevented by finerenone administration at the time of IR.

Conclusion: Altogether, our data shows that finerenone is able to prevent AKI induced by IR as well as the chronic and progressive deterioration of kidney function and structure.

J. Barrera-Chimal: None. A. Le Mercier: None. S. El-Moghrabi: None. P. Kolkhof: A. Employment; Modest; Employee at BayerPharmaAG. F. Jaisser: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Part of the work was supported by a grant from Bayer.

Funding: No

Funding Component: P299

Thymosin Beta15 is a Novel Precursor of N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP)

Tang-Dong Liao, Cesar Romero, Nitin Kumar, Nour-Eddine Rhaleb, Oscar Carretero, Henry Ford Hosp, Detroit, MI

N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) is a natural tetrapeptide with anti-inflammatory and antifibrotic properties that is released from its precursor thymosin β4 (Tβ4). At present, Tβ4 is the only identified Ac-SDKP precursor with no other candidates reported. Our search of National Center for Biotechnology Information (NCBI) database by Basic Local Alignment Search Tool (BLAST) revealed that Tβ15 has similar N-terminal amino acid structure as Tβ4. We hypothesize that in addition to Tβ4, Tβ15 may also be a precursor of Ac-SDKP. To test our hypothesis we used wild-type (WT) and Tβ4 global knockout mice (Tβ4KO). We detected the presence of Ac-SDKP in various organs (pmol/mg protein) in both WT and Tβ4KO mice, including lymph node (459±35 vs 234±37), thymus (241±36 vs 179±24), heart (95±14 vs...
71±10), kidney (90±19 vs 56±11), testis (55±16 vs 55±17), spleen (57±10 vs 16±1) and liver (49±16 vs 18±2). We also measured urinary Ac-SDKP excretion before and after angiotensin converting enzyme inhibitor (ACEi) treatment. In both group of mice we determined the basal Ac-SDKP excretion (ng/24hrs) and that was significantly increased with ACEi treatment in both WT (36±8 vs 117±17) and Tβ4KO (22±3 vs 36±3), with the increase being lower in Tβ4KO mice. Immunohistochemistry analysis of Tβ15 in the kidney revealed positive cells located in the thick ascending limb, distal tubule, macula densa and collecting duct in both groups of mice. Interestingly, we found that in Tβ4KO mice thymus weight was higher than in WT mice, while there was no difference between the groups with regard to heart and kidney weight. We concluded that Tβ15 is a potential novel precursor of Ac-SDKP.

T. Liao: None. C. Romero: None. N. Kumar: None. N. Rhaleb: None. O. Carretero: None.

Funding: No
Funding Component: P300

Cardiac Ly6C<sub>high</sub> Monocyte Accumulation and Remodeling Depend on the Mineralocorticoid Receptor in Endothelial Cells

Achim Lother, Aurelia Hübner, Ingo Hilgendorf, Tilman Schnick, Martin Moser, Christoph Bode, Lutz Hein, Univ of Freiburg, Freiburg, Germany

Introduction
Inflammation is a key driver for the development of cardiac fibrosis and diastolic dysfunction. Aldosterone promotes the expression of adhesion molecules and vascular inflammation. Thus, the goal of the present study was to examine the significance of endothelial MR for pressure overload induced cardiac inflammation and remodeling.

Methods and results

Mice with endothelial cell-specific deletion of the mineralocorticoid receptor (MR<sub>Cdh5Cre</sub>) were generated using the Cre/loxP system. MR<sub>Cdh5Cre</sub> and Cre-negative littermates (MR<sub>wildtype</sub>) underwent transverse aortic constriction (TAC, n=5-7 per group).

After two weeks of pressure overload echocardiography revealed diastolic dysfunction in MR<sub>wildtype</sub> (mitral valve E acceleration time TAC 15.7 ± 0.5 vs. sham 12.8 ± 0.4 ms, P<0.05) but not in MR<sub>Cdh5Cre</sub> mice (TAC 11.2 ± 0.6 vs. sham 12.2 ± 0.9 ms, n.s.).

Cardiac hypertrophy (ventricle weight 143.2 ± 5.2 vs. MR<sub>wildtype</sub> 167.3 ± 6.7 mg, P<0.001) and interstitial fibrosis (sirius red stained area 8.2 ± 4.7 vs. MR<sub>wildtype</sub> 13.5 ± 4.5 %, P<0.05) following TAC were attenuated in MR<sub>Cdh5Cre</sub> mice. mRNA expression of atrial natriuretic peptide (Nppa, 2429 ± 1230 vs. MR<sub>wildtype</sub> 7051 ± 3182 copies/10<sup>4</sup> copies Rps29, P<0.01) or the fibrosis marker gene collagen 1a1 (Col1a1, 256 ± 89 vs. MR<sub>wildtype</sub> 432 ± 165 copies/10<sup>4</sup> copies Rps29, P<0.05) as determined by qRT-PCR confirmed these findings.

Cardiac leukocytes were quantitatively analyzed by fluorescence assisted cell sorting using specific antibodies. Numbers of CD45<sup>+</sup> leukocytes were similarly increased after TAC in the hearts of both genotypes (MR<sub>Cdh5Cre</sub> 3840 ± 443 vs. MR<sub>wildtype</sub> 4051 ± 385 /mg tissue, n.s.). Subtype analysis revealed a shift towards CD45<sup>+</sup> CD11b<sup>+</sup> F4/80<sup>low</sup> Ly6C<sup>high</sup> monocytes vs. CD45<sup>+</sup> CD11b<sup>+</sup> F4/80<sup>high</sup> Ly6C<sup>low</sup> macrophages in the heart of MR<sub>wildtype</sub> (TAC 20 ± 6 vs. sham 4 ± 1 % of CD45<sup>+</sup> CD11b<sup>+</sup>, P<0.05) but not of MR<sub>Cdh5Cre</sub> mice (TAC 6 ± 2 vs. sham 3 ± 1 % of CD45<sup>+</sup> CD11b<sup>+</sup>, n.s.).

Conclusion
MR deletion from endothelial cells ameliorates left ventricular remodeling and diastolic dysfunction after pressure overload. The protective effect of endothelial MR deletion is associated with a shift towards less pro-inflammatory Ly6C<sub>high</sub> monocytes and more reparative Ly6C<sub>low</sub> macrophages.
Renal Expression of Collectrin (TMEM27) is Downregulated During Angiotensin II-induced Hypertension

Sylvia Cechova, Pei-Lun Chu, Joseph Gigliotti, Thu H. Le, Univ of Virginia, Charlottesville, VA

Collectrin (Tmem27) is transmembrane glycoprotein with homology to ACE-2, but lacks any catalytic domain. It plays a key role as a chaperone of amino acid transporters, and is abundantly expressed in the kidney in the proximal tubules and collecting duct. Deletion of collectrin in the mouse results in hypertension (HTN) at baseline and augmented salt-sensitivity that are associated with decreased renal nitric oxide and increased superoxide levels. During high salt diet, renal expression of collectrin is upregulated, suggesting an adaptive homeostatic response to salt loading. Here, we queried whether the expression of collectrin is regulated by angiotensin II (Ang II). Wild-type 129S6 mice were made hypertensive with Ang II osmotic minipump @ 600 ng/kg/min x 2 weeks, and were compared to age-matched untreated WT 129 mice. Shown in Fig. 1, renal mRNA expression of collectrin is significantly reduced after 2 weeks of Ang II (Panel A).

Immunostaining shows collectrin protein level is also significantly diminished to near undetectable level (Panel B). We show for the first time that Ang II regulates the expression of collectrin, suggesting that the action of Ang II on blood pressure may be mediated, in part, through the downregulation of collectrin. Further studies are needed to determine the effect of AT1 and AT2 receptor signaling on renal expression of collectrin during Ang II-HTN in vivo.
assessed by levels of inflammatory markers angiotensin II (Ang II), a powerful vasoconstrictor, and serum adiponectin. We previously demonstrated a marked increase in levels of inflammatory markers in humans and mice with hypertension, obesity, and diabetes, and hypothesize that a high-BMI subject would present with increased levels of angiotensin II, leptin, and TNF-α, as well as decreased adiponectin. Methods: Serum adiponectin, Ang II, leptin, and TNF-α were assayed in female Appalachian subjects. Linear regression analysis was used to analyze the relationship between BMI, leptin, adiponectin, and Ang II. Nonlinear regression was used to determine the odds ratio and confidence intervals. The effect on murine pre-adipocytes was also measured. Results: Lipidomic analysis revealed a significant increase in Ang II among high-BMI females (50-72) compared to lower-BMI subjects (32-45) (p<0.05). Treatment of murine pre-adipocytes with Ang II decreased serum adiponectin levels and increased adipogenesis by 70% (p<0.05), implicating both Ang II and oxidative stress as factors in the pathogenesis of BMI-related disease. Serum leptin and TNF-α were significantly increased in high-BMI subjects (p<0.05) compared to lower-BMI subjects, while adiponectin levels were decreased (p<0.05) in high-BMI subjects compared to lower-BMI subjects. Discussion/Conclusions: Increased BMI in Appalachian females correlates with an increase in Ang II level, serum TNF-α, and leptin expression, and a decrease in serum adiponectin. This represents a novel mechanism by which high-BMI females with controlled blood pressure remain sensitive to the development of atherogenesis, vascular dysfunction, and metabolic syndrome.


Funding: No

Funding Component: P303

Cilostazol Attenuates Angii-induced Cardiac Fibrosis in Mice

Yuka Okuyama, Haruhito A Uchida, Ryoko Umebayashi, Yuki Kakio, Hidemi Takeuchi, Michihiro Okuyama, Jun Wada, Okayama University, Okayama, Japan

Background: Cilostazol, a phosphodiesterase-3 inhibitor, plays vasoprotective roles such as an improvement of endothelial function, a vasodilatation and a suppression of proliferation of vascular smooth muscle cells. The aim of study was to investigate a cardioprotective effect of cilostazol.

Method: Male apolipoprotein E deficient mice (8-12 weeks old) were fed with either normal chow diet or cilostazol-containing (0.1% wt/v) diet. After 1 week of cilostazol administration, mice were infused subcutaneously with either angiotensin II (AngII, 1,000 ng/kg/min, n = 16 - 19) or saline (n = 5 - 6) by osmotic minipumps for 4 weeks.

Results: AngII equivalently increased systolic blood pressure, irrespective of cilostazol administration. Cilostazol had no effect on serum cholesterol concentrations, triglycerides, high-density lipoprotein-cholesterol, body weights, heart rates, and systolic blood pressures. AngII increased heart weight but was attenuated by cilostazol administration (6.7±0.8 to 6.0±0.7 mg/gBW, p < 0.05). Cilostazol prevented both perivascular and interstitial cardiac fibrosis induced by AngII (p < 0.05, each). Quantitative real-time PCR revealed that mRNA expressions of Ctgf, Collagen I, Collagen III, Tgf-β, Hgf and Spp-1 increased by AngII infusion but were attenuated by cilostazol administration (p < 0.05). Immunohistochemical analysis demonstrated that AngII administration enhanced OPN expression in heart but was suppressed by cilostazol administration.

Further, to investigate the mechanism, human
cardiac myocytes were cultured and stimulated with AngII (1x10^{-7} M). Co-treatment of Cilostazol (1x10^{-7} to 1x10^{-5} M) attenuated AngII-induced increase of Spp-1 gene expression in dose-dependent manner. This effect was mimic by a treatment with forskolin, which was diminished by co-treatment with H-89.

Conclusion: Cilostazol attenuated AngII-induced cardiac fibrosis in vivo. Cilostazol attenuated AngII-induced increment of Spp-1 gene expression through cAMP-PKA dependent pathway.


Funding: No

Funding Component: P304

A Sympathetic-cholinergic Pathway Performs a Connection Between Brain and Spleen, to Prime Immune System and Induce Arterial Hypertension

Daniela Carnevale, Sapienza Univ of Rome at IRCCS Neuromed, Pozzilli (IS), Italy; Marialuisa Perrotta, IRCCS Neuromed, Pozzilli, Italy; Fabio Pallante, Lorenzo Carnevale, Giuseppe Cifelli, Valentina Fardella, Roberta Iacobucci, Stefania Fardella, IRCCS Neuromed, Pozzilli (IS), Italy; Giuseppe Lembo, Sapienza Univ of Rome at IRCCS Neuromed, Pozzilli, Italy

It is now widely recognized that immune system has a crucial role in hypertension. Various studies have demonstrated that the activation of adaptive immunity, and in particular of T cells, is a crucial moment in the onset and maintaining of hypertension induced by various stimuli in mice. Our previous studies have shown that hypertensive stimuli couple the sympathetic nervous system to determine the activation of splenic immune system. However, how the brain-to-spleen connection is realized in hypertension remains unknown. In this study we demonstrate that mice subjected to various hypertensive stimuli (AngII, DOCA-salt) show an increase of sympathetic nervous activity recorded in vivo in the splenic nerve (Firing Frequency: AngII 131±17 vs Veh 30±10 spikes/10 min, p<0.001). We also show how the sympathetic pathway induced by pro-hypertensive stimuli has its origin in the brain, converging into the spleen through a cholinergic-sympathetic connection that is realized through the vagus-splenic nerve drive and mediated at the molecular level by cholinergic nicotinic receptors at the level of celiac ganglion. In fact, we show that in celiac vagotomized mice, i.e. mice subjected to a procedure inhibiting vagal efferents but not central afferents, the splenic nervous drive induced by AngII was absent (AngII+VagX 21±4 vs AngII+sham 148±29 spikes/10 min, p<0.001). The same result was shown in α7 cholinergic nicotinic receptor KO mice, a receptor typically expressed by neurons in the peripheral ganglia (α7nAChR KO AngII 43±8 vs WT AngII 141±27 spikes/10 min, p<0.01). Moreover, we found that this cholinergic-sympathetic pathway was necessary to allow the activation of T cell costimulation and egression upon hypertensive challenges. Our results highlight a cholinergic-sympathetic pathway played by vagus-splenic nerves and responsible for immune system activation in response to hypertensive stimuli. We believe our results are significant because they reveal a previously unknown sympathetic pathway in hypertension for the first time. The brain-to-spleen connection realized through a cholinergic-sympathetic nervous drive that resembles the cholinergic anti-inflammatory pathway identified by immunologists in endotoxemia.

Sympathetic Activation Elicited by Bilateral Carotid Occlusion Attenuates Cytokine Release in Conscious Endotoxemic Rats

Fernanda Brognara, Jaci A Castania, Daniel P Dias, Rubens Fazan Jr., Fernando Q Cunha, Ribeirão Preto Medical Sch, Ribeirão Preto, Brazil; Kaushik P Patel, Univ of Nebraska Medical Ctr, Omaha, NE; Luis Ulloa, New Jersey Medical Sch, Newark, NJ; Alexandre Kanashiro, Helio C Salgado, Ribeirão Preto Medical Sch, Ribeirão Preto, Brazil

Our previous studies suggest that carotid occlusion can induce a sympathetic signal able to modulate the immune system. Here, we analyze whether bilateral carotid occlusion (BCO) affect the innate immune response to bacterial lipopolysaccharide (LPS) in endotoxic rats. In order to prevent the neuronal alterations induced by anesthesia, we performed our studies of BCO in conscious rats. Wistar rats were implanted with pneumatic cuffs around the common carotid arteries for BCO. Femoral and peritoneal catheters were also inserted for blood pressure recording and LPS administration. Rats were randomly assigned to the following groups: Saline, LPS + SHAM (LPS in the presence of the occluders but without occlusion) and LPS + BCO (LPS combined with bilateral carotid occlusion). BCO was performed for 20s in conscious awake rats, right before LPS (5.0 mg/kg) or saline (control) administration. Plasma and spleen samples were collected at 90 min after LPS or saline administration. As compared to baseline arterial pressure, BCO produced a peak response in mean arterial pressure of 52 ± 3 mmHg, confirming the sympathetic activation. BCO significantly attenuated TNF-α and IL-1β plasma levels as compared to those in SHAM endotoxemic rats [TNF: 1319 ± 388 (n = 6) vs. 583 ± 138 pg/mL (n = 8), P = 0.03; IL-1β: 2203 ± 256 (n = 5) vs. 1086 ± 157 pg/mL (n = 9), P < 0.001]. BCO also significantly reduced the TNF-α levels in the spleen [5.1 ± 1.3 (n = 7) vs. 1.7 ± 0.4 pg/mg tissue (n = 8), P = 0.005]. By contrast, BCO did not significantly change IL-6 and IL-10 levels in plasma [IL-6: 5609 ± 352 (n = 7) vs. 5879 ± 375 pg/mL (n = 10), P = 0.703; IL-10: 2417 ± 354 (n = 5) vs. 2068 ± 298 pg/mL (n = 9), P = 0.408] or in the spleen [IL-6: 15 ± 3 (n = 7) vs. 14 ± 2 pg/mg tissue (n = 10) P = 0.776; IL-10: 1.6 ± 0.2 (n = 7) vs. 1.3 ± 0.2 pg/mg tissue (n = 9), P = 0.475]. Moreover, the IL-1β level in the spleen was not affected by BCO [54 ± 12 (n = 6) vs. 28 ± 5 pg/mg tissue (n = 9), P = 0.058]. These findings indicate that sympathetic activation by BCO in conscious rats attenuates the pro-inflammatory cytokines release in the endotoxemic model induced by LPS without affecting anti-inflammatory cytokine IL-10.


Inflammatory Biomarkers Participate in Physiopathology of Hypertensive Crisis

Jose F Vilela-Martín, Days Oliveira-Andrade, Luciana N Cosenso-Martin, Michele L Gregorio, Moacir F Godoy, Juan C Yugar-Toledo, Doroteia R Souza, State Medical Sch in Sao Jose Rio Preto (FAMERP), Sao Jose do Rio Preto, Brazil

Background: Recent evidence suggests the existence of an underlying inflammation process to vascular disease associated with chronic high blood pressure (BP), playing an important role in the pathophysiology of hypertension (HT). However, few studies show
the participation of the inflammatory process in the pathophysiology of acute BP elevation.

**Objectives:** To identify clinical and metabolic profile of presentation of hypertensive crisis (HC) divided into hypertensive urgency (HUrg) and emergency (HEmerg); 2 - To assess the involvement of inflammatory cytokines: IL-1β, IL-6, IL-8, IL-18, TNF-α and anti-inflammatory IL-10 in subjects with HC.

**Methods:** We studied 274 individuals: 74 normotensive (NT), 74 controlled hypertensive (CHT), 50 HUrg and 78 HEmerg. Serum levels of cytokines were made by MULTIPLEX and ELISA techniques. Analysis of variance was used to compare the groups, with significant p-value <0.05.

**Results:** The diastolic BP and heart rate were greater in the HC group (120mmHg and 85bpm, respectively) compared to the CHT group (75mmHg and 68 bpm, respectively; p<0.05). Individuals with HEmerg were older. Glycaemia was significantly higher in the HEmerg group (113mg/dL) in comparison to NT and CHT (91mg/dL and 98mg/dL, respectively; p<0.05). HDL-c was significantly lower in HEmerg group compared to the NT group (p=0.0088). Potassium levels were lower in HEmerg group compared to the other groups NT, CHT and HUrg (p=0.0118, p=0.036 and p=0.036; respectively). All measured cytokines were significantly greater in subjects who had HC compared to NT and CHT groups. Logistic regression showed that IL-1β, IL-6, IL-8 and IL-18 were predictors for HC development with odds ratios of: 4.29 (2.01 - 9.19), 11.02 (3.98 - 30.45), 22.56 (7.99 - 63.64) and 3.85 (1.85 - 8.02), respectively.

**Conclusions:** The levels of inflammatory cytokines (IL-1β, IL-6, IL-8 and IL-18) and anti-inflammatory (IL-10) are higher in HUrg and HEmerg groups compared to NT and CHT groups. This suggests the involvement of inflammatory cytokines in the pathogenesis of acute hypertensive event.

**J.F. Vilela-Martin:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Support FAPESP. **D. Oliveira-Andrade:** None. **L.N. Cosenso-Martin:** None. **M.L. Gregorio:** None. **M.F. Godoy:** None. **J.C. Yugar-Toledo:** None. **D.R.S. Souza:** None.

**Funding:** No

**Funding Component:** P307

---

**Effect of Bradykinin Receptor Antagonism on ACE Associated Angioedema**


The B2 receptor antagonist icatibant is approved for treatment of attacks of hereditary angioedema. Icatibant has been reported to decrease time-to-resolution of ACE inhibitor-associated angioedema in one study of European patients. Patients with ACE inhibitor-associated angioedema (defined as swelling of lips, tongue, pharynx or face during ACE inhibitor use and no swelling in the absence of ACE inhibitor use) were randomized within six hours of presentation to SQ icatibant 30 mg or placebo at zero and six hours later. Patients assessed severity of swelling using a visual analog scale serially following study drug administration or until discharge. Thirty-one patients were randomized to and received treatment, with 13 receiving icatibant and 18 receiving placebo. One patient randomized to icatibant did not complete the visual analog scale and was excluded. Two-thirds of patients were African American and two-thirds were women. Time-to-resolution of symptoms was similar in placebo and icatibant treatment groups [p=0.19 for the primary symptom (Figure) and p>0.16 for individual symptoms of face, lip, tongue, or eyelid swelling]. Frequency
of administration of H1 and H2 blockers, corticosteroids, and epinephrine was similar in the two treatment groups. Lip swelling was more severe in blacks (p=0.03), while tongue swelling was more severe in whites (0.02). White patients were significantly more likely to receive epinephrine (p=0.01). Time-to-resolution of symptoms was similar in blacks and whites. This study does not support the clinical efficacy of administration of a B2 receptor blocker in black or white American patients with ACE inhibitor-associated angioedema.

C.E. Ramirez: None. B.T. Straka: None. J.B. Byrd: None. E. Stone: None. A. Woodard-Grice: None. N. Hui: None. C. Yu: None. A. Banerji: None. N.J. Brown: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; UL1 RR024975, R01HL079184, Investigator-Initiated Research Program from Jerini AG/Shire Pharmaceuticals, Inc, New Haven Pharmaceuticals. G. Consultant/Advisory Board; Modest; Novartis Pharmaceuticals, Alynym Pharmaceuticals.

Funding: No


The study objective was to study the effect of melatonin on blood pressure (BP) in both younger and older normotensive and prehypertensive individuals. Preliminary data available at the time of abstract submission are presented for 12 participants (10 ≤ 50 years old; two > 60). free of known hypertension or diabetes. All subjects had office and 24 hour ambulatory BP measured before and after dosing of 9 mg controlled release melatonin for six weeks. Subjects are described in Table 1. Full data analysis for all 30 pilot subjects, including 12 older and 10 control subjects, will be available for the presentation. Table 2 shows results comparing baseline and follow-up BP. Mean 24 hour SBP/DBP, as well as ambulatory daytime SBP/DBP, were significantly lower following melatonin administration.

In conclusion, these findings suggest possible future utility of melatonin for lowering BP in both younger and older non-hypertensive individuals.
Safety, Tolerability and Pharmacokinetic Data of the Novel Orally Active Formulation of Angiotensin-(1-7), Hydroxypropyl-β-cyclodextrin/ Ang-(1-7), in Healthy Volunteers- A Randomized Double-blinded Controlled Pilot Study

Janaina Koenen, Marilene Oliveira, UFMG, Belo Horizonte, Brazil; Daniele Hamamoto, Labfar Pesquisa e Serviços Ltda, Belo Horizonte, Brazil; Sérgio H Santos, Ruben Sinisterra, UFMG, Belo Horizonte, Brazil; Frederico B. Sousa, UNIFEI, Itajubá, Brazil; Antônio Ribeiro de Oliveira Junior, Rodrigo Foscolo, Robson A Santos, UFMG, Belo Horizonte, Brazil

Angiotensin-(1-7) is an endogenous peptide of the renin-angiotensin system in humans. It has several properties, which make it of great interest for healthcare, such as in systemic/pulmonary hypertension treatment, insulin resistance improvement, reduction of visceral obesity and cardiac remodeling and arrhythmias. In this randomized double-blinded controlled phase I study we aimed to determine safety, tolerability, and pharmacokinetic properties of the new drug, angiotensin-(1-7) included in Hydroxypropyl-β-cyclodextrin [Ang(1-7)/HPβCD]) in healthy adult volunteers, who were recruited in accordance to inclusion and exclusion criteria. Thirty-two volunteers (18-40 years-old) were admitted to a hospital clinical research ward and remained under observation for two days, during which they received a single oral dose of Ang(1-7)HPβCD (equivalent to 0.35, 1.75 or 7.0 mg of the peptide) or placebo (N=8/group). Vital signs and side effects were recorded according to the study protocol and blood samples were collected to obtain the pharmacokinetic profile of Ang-(1-7)using LC/MS/MS. The oral administration of the novel compound Ang-(1-7)/HPβCD caused a dose-dependent elevation in the Ang-(1-7) plasma levels with a Tmax of 6.5±0.6 hours. The medians of the area under the curve for the placebo, 0.35, 1.75 and 7.0 mg doses were (in pg/mL/24 hours): 837.5±139.8, 1094.4±224.3, 1415.0±187.7 and 1719.4±304.9, respectively. The Cmax for the 0.35, 1.75 and 7.0 mg doses were 26.1±3.17 pg/mL, 30.7±4.18pg/mL and 44.1±7.42 pg/mL, respectively. We did not find any statistically significant differences among the clinical and laboratorial parameters before and after the study. There were few mild side effects reported in the study, not related to the dose:


Funding: No

Funding Component: P309
two volunteers reported headache (one in the placebo group and one in the 0.35mg group) and three had dizziness and sweating in the orthostatic position (one in the 0.35 and two in the 1.75mg group). Our data show that the novel Ang-(1-7)/HPβCD formulation allow the absorption of Ang-(1-7), and is safe and well tolerated. Our results open new perspectives for future clinical trials with Ang-(1-7)/HPβCD for the treatment of arterial hypertension and other conditions.


Funding: No
Funding Component: P310

Reduction of Blood Pressure and Improvement of Heart Rate Variability in Hypertensive Cohort Associated With Use of a Closed Loop Neurotechnology

Hossam A Shaltout, Catherine L Tegeler, Charles H Tegeler, Wake Forest Univ, Sch of Med, Winston Salem, NC

Background: Hypertension and impaired autonomic function increases the risk of many cardiovascular disorders. Disturbed central control of cardiovascular regulation due to chronic stress, anxiety, or other causes may result in hypertension and impaired heart rate variability (HRV). High-resolution, relational, resonance-based, electroencephalographic mirroring (HIRREM) is a noninvasive, closed-loop, acoustic stimulation neurotechnology in which scalp sensors identify dominant brain frequencies, and translate them in real time into audible tones, to support auto-calibration and self-optimization of brain rhythms. Improved HRV after HIRREM is reported with hot flashes, and POTS. Method: We studied effects of HIRREM on BP and autonomic function in subjects with BP >130/90 at baseline enrolled in an ongoing, IRB-approved, open label feasibility study evaluating HIRREM for diverse neurological/psychophysiological disorders. Ten participants (5 female), mean (SD) age 47.2 (20.1), received 17.7 (5.9) HIRREM sessions over 28.6 days (20.1). Data were collected before, and 1-2 weeks after HIRREM completion.

Results: Systolic arterial pressure was reduced (from 152 (17) to 136 (19) mmHg, p=0.018), and diastolic reduced (from 97.2 (8) to 81 (5) mmHg, p<0.001), with no change in heart rate. HRV measured as SDNN increased from 42 ± 7 to 57 ± 9.7 ms (p=0.049), and baroreflex sensitivity measured by sequence method was significantly improved from 10.6 (8) to 16.3 (11) ms/mmHg (p=0.001). Symptoms of insomnia (Insomnia Severity Index) were reduced (from 8.1 (7) to 3.3 (3), p=0.009), with a trend for reduction in anxiety (GAD-7, from 7.2 to 2.2 p=0.06). There were no adverse events or dropouts. Conclusion: These results are the first to suggest both cardiovascular and behavioral benefits for HIRREM in a hypertensive cohort. Effects may be related to reduced insomnia and anxiety, but could reflect reduced allostatic load, and improved central autonomic balance, with resulting reduced peripheral sympathetic tone. Further studies are warranted to elucidate mechanism(s) of changes associated with this noninvasive, non-drug intervention.

H.A. Shaltout: None. C.L. Tegeler: None. C.H. Tegeler: None.

Funding: No
Funding Component: P310

Cardiac Sympathetic Afferents Does Not Initiate but Contributes to the Further Development of Hypertension in Spontaneous Hypertensive Rats
Sympatho-excitation plays a critical role in the pathogenesis of hypertension. However, it is unclear what factors initiate and maintain sympatho-excitation in hypertension. Our past studies have confirmed a critical role of cardiac sensory nerve endings that mediate a sympatho-excitatory reflex called the “cardiac sympathetic afferent reflex” (CSAR) in the setting of heart failure. However, whether/when the CSAR is activated and contributes to the development of hypertension remains unclear. To address this issue, we chronically abolished the CSAR by epidural application of a selective afferent neurotoxin, resiniferatoxin (RTX) at the level of the T1-T4 dorsal root ganglia (DRGs) by destroying TRPV1-expressing neuronal soma in 8-week and 16-w old spontaneous hypertensive rats (SHR). Conscious blood pressure was monitored before (baseline) and during 2 months post RTX using radio telemetry. As shown in Figure 1A, in early-hypertensive (8-w old) SHR rats, there was no difference in mean arterial pressure (MAP) between vehicle and RTX groups until 3 weeks post intervention. At that time, MAP in vehicle-treated SHR rats continued to increase whereas this increase was largely abolished in the RTX-treated group. In the established (16-w old) SHR rats (Figure 1B), treatment with RTX immediately reduced MAP by ~15 mmHg, which was maintained for the 2-month recording period. These data strongly suggest that although CSAR does not initiate hypertension at the early stage in SHR, it contributes to the further development of hypertension in the mid/late stages. These data support a potential novel therapy possibly involving cardiac afferents.
MC4R agonist reduces blood glucose and prevents bradycardia in diabetic rats, we used a novel compound with high affinity to MC4R. Male 12-week-old Sprague-Dawley rats (n=5/group) were instrumented with telemetry probes for determination of mean arterial pressure (MAP) and heart rate (HR) 24-hrs/day and an intracerebroventricular (ICV) cannula was placed in the brain lateral ventricle for continuous infusion of the selective MC4R agonist PL6214 (2.5 μg/hr) or MTII (a non-selective MC3/4R agonist, 10 ng/hr) via osmotic minipump. Induction of diabetes caused hyperphagia (20±1 to 32±2 g), hyperglycemia (89±3 to 494±44 mg/dl) and bradycardia (-47 bpm). Chronic infusion of PL6214 for 11 days transiently reduced food intake which returned to diabetic values by day 6 after starting the infusions, whereas chronic infusion of MTII caused a reduction in food intake lasting only 3-4 days. PL6214 reduced blood glucose by 63% on day 2 and 16% by day 11 of infusion, and prevented further bradycardia induced by diabetes (-35±14 bpm on the last day of infusion). Chronic MTII infusion reduced blood glucose by 31% on day 2 and by day 6 glucose levels had already returned to values observed before treatment was started. MTII infusion did not attenuate the bradycardia (-99±13 bpm on the last day of infusion). Diabetes did not alter MAP, while the MC4R agonist increased MAP by 5±1 mmHg compared to control values. These results indicate that selective activation of MC4R agonist attenuates bradycardia and hyperglycemia in type 1 diabetes, and may provide a new strategy for treatment of diabetes. (P20GM104357, NHLBI-P01HL51971, AHA-SDG5680016 and Palatin Technologies).


Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P313

**Alterations in Notch Signaling in Diabetic Coronary Microvascular Smooth Muscle and Endothelial Cells**

**Patricia McCallinhart**, Brenda Lilly, Aaron J Trask, Nationwide Children's Hosp, Columbus, OH

Notch signaling between vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) is crucial during normal vascular development and homeostasis and may contribute to vasculopathies. Notch signaling is known to regulate VSMC phenotypic mechanisms, including proliferation and differentiation. We previously reported differences in VSCM Notch3 and EC Jagged1 mRNA expression, as well as a de-differentiated VSMC phenotype, in diabetic coronary resistance microvascular (CRM) cells as a potential mechanism for the inward remodeling observed in those vessels. The current study tested the hypothesis that VSMC-EC dependent Notch signaling accounts for phenotypic changes in VSMCs that may promote adverse remodeling observed in diabetic CRMs. Low-passage primary coronary VMSCs were isolated from normal (n=6) and type 2 diabetic db/db mice (n=4) and were either cultured alone or co-cultured with normal or type 2 diabetic human coronary microvascular endothelial cells (hCMECs), respectively. Notch2 expression was significantly attenuated in diabetic CRM VSMCs in both mono- and co-culture conditions (0.53±0.12 vs. 1.00±0.06, p<0.01 and 0.59±0.09 vs. 0.94±0.04, p<0.01, respectively). There were no significant differences in Hes1, and Hrt3 was increased in only normal VSMCs that were co-cultured with ECs. We further found that
proliferation inhibitor p53 expression was significantly lower in diabetic CRM VSMCs in when co-cultured with ECs (0.78±0.15 vs. 1.34±0.13, p<0.01), and Smad3 was significantly reduced in diabetic co-cultured CRM VSMCs (0.90±0.02 vs. 1.14±0.06, p<0.05). Collectively, these data implicate that Notch signaling through p53 and Smad3 may account for phenotypic alterations of diabetic CRM VSMCs.

P. McCallinhart: None. B. Lilly: None. A.J. Trask: None.

Funding: No
Funding Component: P314

**Urinary Renin and Kidney RAS Activation in Mice with Diabetic Kidney Disease**

**Johannes Rein**, Patricia Valles, Jan Wysocki, Minghao Ye, Northwestern Univ, Chicago, IL; Mariam Afkarian, Univ of Washington, Seattle, WA; Daniel Batlle, Northwestern Univ, Chicago, IL

RAS is overactive in kidneys from patients with diabetic nephropathy (DN), but circulating plasma renin activity (PRA) is usually low. This is known as the renin-paradox. We evaluated juxtaglomerular (JGA), tubular and urinary renin, as a potential source of local RAS activation, to gain some understanding of this paradox.

Mice with STZ induced diabetes were used which had mild albuminuria and glomerular mesangial expansion consistent with early DN. Renin expression in the JGA and in the collecting tubule (CT) was evaluated by immunohistochemistry. IF was used to localize renin within CT cells. Proximal tubular renin was evaluated by RT-real time PCR in microdissected proximal tubules (PT). Urinary renin and Ang II were measured by ELISA.

Urinary Ang II was increased (37.8±11.4 vs. 99.0±21.6 pg/mg creat, p<0.05) reflecting an active kidney RAS. Urinary renin was also increased in STZ-treated as compared to controls (Table). In microdissected PTs there were no significant differences in renin mRNA between control and STZ-mice. By immunostaining, renin was localized to principal cells in the CT and the number of renin stained CTs was higher in STZ than in control mice. In sharp contrast, renin staining of the JGA of STZ-mice was significantly reduced as compared to controls.

We conclude that in DN renin expression in the JGA, the physiologic site of renin secretion into the circulation is suppressed, whereas in the CT it is increased. Activation of the kidney RAS, as inferred from increased urinary Ang II, likely occurs as a result of renin of tubular origin rather than from JGA renin. Since PT renin is not increased, the CT may provide the source of tubular renin for RAS activation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups (n)</th>
<th>Mean±SEM (Units)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT Renin</td>
<td>Control (8) vs. STZ (7)</td>
<td>0.4±0 vs. 100±10 (pg/mg creat)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>CT Renin</td>
<td>Control (9) vs. STZ (9)</td>
<td>1±1 vs. 1±1.5 (No. of Renin stained CTs/total area)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>JGA Renin</td>
<td>Control (9) vs. STZ (9)</td>
<td>26±5 vs. 18±6 (percent of Renin stained JGA/total area)</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>


Funding: No
Funding Component: P315

**Vascular AT1 Angiotensin Receptors Do Not Contribute to Albuminuria or Hyperfiltration in Diabetes**

**Matthew A Sparks**, Stacy Johnson, Rishav Adhikari, Edward Diaz, Aaron Kupin, Kat Bendt, Susan Gurley, Thomas Coffman, Duke Univ, Durham, NC
Blockade of the renin angiotensin system (RAS) reduces albuminuria, attenuates hyperfiltration, and slows the progression of diabetic nephropathy (DN) by preventing vasoconstriction and subsequent increases in glomerular hydrostatic pressure. Since RAS blockade disrupts Ang II signaling in all tissues, the specific contribution of vascular actions of AT1 receptors in DN has been difficult to delineate. Therefore, we generated 129 SvEv mice with cell-specific loss of AT1A from VSMCs (SMKOs) using Cre-loxp. To eliminate AT1R from VSMCs, we crossed the SMKO mice with AT1BR-/- mice, lacking the minor AT1B isoform. To study the impact of vascular AT1R in DN, we crossed the AT1B-null SMKOs with mice having the Ins2<sup>C96Y</sup> AKITA mutation, which develop DM1 early. To enhance kidney injury, mice underwent uninephrectomy (UNX) at 11wks. Blood glucose levels were elevated (~500 mg/dL) and similar at 10, 16 and 24wks between the two groups. Prior to UNX, albuminuria was similar between Control AKITA and AT1B-null SMKO AKITA (62±10 Control AKITA versus 107±27 µg/24hrs SMKO AKITA, P=NS). Albuminuria increased with age in both Control Akita and AT1B-null SMKO AKITA but without significant differences between the groups at 16wks (307±106 vs 313±117 µg/24hrs; P=NS) or 24wks (494±236 versus 730±217 µg/24hrs; P=NS), despite a trend toward higher albuminuria in AT1B-null SMKO AKITAs. There was no significant difference in GFR (using FITC-inulin) between non-diabetic Control and AT1B-null SMKO (15.6±1.2 vs 14.8±0.8 µl/min/g BW), and hyperfiltration was observed in both Control AKITA (23.7±2.4 µl/min/g BW; P=0.003) and AT1B-null SMKO AKITA mice (20.7±1.7 µl/min/g BW; P=0.01) relative to their non-diabetic comparators. However, there was no significant difference in GFR between Control AKITA and AT1B-null SMKO AKITA (P=NS). Finally, we measured mRNA levels of putative kidney injury markers by RTqPCR and found no differences in levels of Col1A1, NGAL, or TGFB1 mRNA between Control AKITA and AT1Bnull SMKO AKITA. Our studies indicate that the absence of vascular AT1R responses is not sufficient to reduce albuminuria and prevent hyperfiltration in a mouse model of DN. This suggests that blockade of AT1R in other cell lineages may contribute to beneficial actions of ARBs in DN.


Funding: No

Funding Component: P316

Angiotensin-(1-7) Contributes to the Insulin-sensitizing Effects of Renin-angiotensin System Blockade in High Fat Fed Mice

Amy C Arnold, Penn State Coll of Med, Hershey, PA

Angiotensin-converting enzyme (ACE) inhibitors reduce body weight, lower blood pressure, and improve glucose homeostasis in animal models of the cardiometabolic syndrome. These effects are generally attributed to a reduction in angiotensin (Ang) II formation; however, these therapies also increase circulating levels of Ang-(1-7), a peptide with direct anti-hypertensive and insulin-sensitizing effects. In this study, it was hypothesized that endogenous Ang-(1-7) generation contributes to the beneficial cardiometabolic effects of ACE inhibition. To test this hypothesis, diet-induced obesity was produced in adult male C57BL/6J mice by placing them on a 60% high fat diet for 11 weeks. The Ang-(1-7) mas receptor antagonist A779 (400 ng/kg/min) or saline was given after 8 weeks of the high fat diet by subcutaneous osmotic mini-pumps. Immediately following mini-pump implantation, mice received water containing the ACE inhibitor captopril (50 mg/L)
or plain tap water. Hyperinsulinemic (4 mU/kg/min) euglycemic clamps were performed in conscious, unrestrained vehicle (n=6), captopril (n=6), or captopril plus A779 (n=13) mice at the end of the 3-week treatment period. Blood pressure was measured via an indwelling carotid artery catheter connected to a transducer on the morning of the clamp. Captopril reduced body weight (28±2 vs. 41±2 g vehicle; p=0.001), lowered blood pressure (systolic: 109±6 vs. 144±7 mmHg vehicle; p=0.003), and improved whole-body insulin sensitivity (steady-state glucose infusion rate: 31±4 vs. 16±2 mg/kg/min; p=0.008) in high-fat fed mice. Mas receptor antagonism with A779 attenuated the improvement in insulin sensitivity produced by captopril in high fat fed mice (23±2 mg/kg/min; p=0.042). There was no effect of A779 on the weight loss (32±2 g) or blood pressure lowering effects (111±7 mmHg) of captopril. These findings suggest that the improvement in insulin sensitivity produced by ACE inhibition is at least partly mediated by Ang-(1-7) pathways, and provide new insight into potential mechanisms involved in renin-angiotensin system blockade.

A.C. Arnold: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH HL122507.

Funding: No

Funding Component: P317

Obesity Subtypes and Prevalence, Treatment and Control of Hypertension in HIV Infected and Welltreated Patients

Hong Seok Lee, Belen Nunez, Amrut Savadkar, Ferdinand Visco, Gerald Pekler, Savi Mushiyev, Metropolitan Hosp , New York Medical Coll, New York, NY

Objective: The purpose of this study is to find out the relationship between obesity subtypes and hypertension’s prevalence, treatment and control in HIV population. Background: HIV infected patients may develop abnormalities in their metabolic panel whether due to the viral infection itself or from the antiretroviral therapy. However, the prevalence of hypertension and, treatment and control in subgroups of obesity of HIV infected patients have not been systematically studied. Methods: Four hundred seventy two HIV infected patients were identified in the registry of Metropolitan Hospital Center from January 2012 to July 2015. Retained cases were assigned as defined by the National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATP) III definition of metabolic syndrome. Results: The prevalence of hypertension in MHNW (metabolically healthy normal weigh), MONW (metabolically obese normal body weight), MOO (metabolically obese obese body weight) and MHO (metabolically healthy obese body weight) obese group was 16.1%, 24.2%, 21.8% and 14.9% respectively. Treatment rate of hypertension was 61.2%, 56%, 67% and 55% among MHNW, MONW, MOO and MHO obese each group. Control of hypertension was 70.1%, 61.2%, 64.6% and 84.5%. Mean systolic blood pressure were 128.1±16, 115.1±14, 123.1±11 and 127.1±13 mmHg (p = 0.152). Diastolic blood pressure were 71.9±10, 78.1±11, 77.1±9.8 and 72.1±5 mmHg (p = 0.043) respectively. Male sex in MONW [OR: 8.511, 95% CI: 1.447-9.208] was found to be the risk factor for controlled blood pressure. In MONW, lower LDL (<100 mg/dl) showed statistically non-random association with controlled blood pressure as compared to LDL over 100 (mg/dl) [ (OR): 1.512, 95% CI: 1.303-2.116]. Conclusions: In HIV infected patients, MONW is more prone to have higher prevalence of hypertension and uncontrolled blood pressure. It suggests that this subtype require more intensive blood pressure management, moreover, female in this group...
needs to be closely monitored because it is a significant risk factor for uncontrolled blood pressure. Also, Higher LDL in MONW might be a risk factor for uncontrolled blood pressure which should be followed up closely. Therefore, strategies to diminish cardiovascular complications than MONW.


Funding: No
Funding Component: P318

miR-155 Induces Inflammation by Polarizing M1 Macrophages in a TLR3-induced Preeclamptic Mouse Model

Valorie L. Chiasson, Baylor Scott and White Health/Texas A and M Health Science Ctr, Temple, TX; Kelsey R. Bounds, Derek Charles, Giuseppina Dusio, Baylor Scott and White Health, Temple, TX; Richard P. Tobin, M. Karen Newell Rogers, Piyali Chatterjee, Baylor Scott and White Health/Texas A and M Health Science Ctr, Temple, TX

Placental microRNA (miRNA) expression is known to be dysregulated during preeclampsia (PE), a hypertensive pregnancy disorder but the role it plays in the pathogenesis of PE is currently unknown. We have previously demonstrated that placental miR-155 expression is upregulated (P-PIC: 3.15 fold, p<0.05 vs. controls) in a TLR3-induced PE mouse model similar to PE patients. In addition, poly I:C (PIC) treatment significantly increased systolic blood pressure (SBP) at gestational day 17 in P-PIC WT (147±4.5 mmHg) compared to P WT mice (103±2.7 mmHg), but did not have any effect in P-PIC miR-155 KO mice (101±2 mmHg). In PE there is an increase in the number of total and activated macrophages. M1 macrophages display the capacity to shift T cell responses toward a TH1 while M2 macrophages promote a TH2 response. We hypothesized that TLR3-induced upregulation of miR-155 contributes to hypertension in part by polarizing the macrophages to a M1 phenotype and these effects will be attenuated in P-PIC miR-155 KO mice. Placental flow cytometry analyses demonstrate that administration of poly I:C induced CD45(+) CD11b(+) macrophages in P-PIC WT mice which is attenuated in P-PIC miR-155 KO mice. In addition, placental classical ‘M1’ macrophages CD45(+) CD11b(+) CD86(+) CD206(-) were increased and alternate ‘M2’ macrophages CD45(+) CD11b(+) CD206(+) CD86(-) were decreased in P-PIC WT mice compared to controls. Interestingly, administration of poly I:C did not change M1 macrophages but increased M2 macrophages in P-PIC miR-155 KO mice. P-PIC WT mice exhibited increased placental expression of additional M1 markers (NOS2, IFNg) and decreased expression of M2 markers (Arg1, IL-10) compared to controls by qRTPCR but not in P-PIC miR-155 KO mice. The above observations are also consistent with our splenic flow cytometry and qRTPCR studies. Based on our results, miR-155 activation induces inflammation in part by increasing M1 macrophages thus contributing to hypertension. Targeting the innate immune system by inhibition of monocyte/macrophages may have beneficial cardiovascular effects in women with PE.


Funding: Yes
Funding Component: National Center
P319

Chronic Ischemia Induces Global Changes in Placental Epigenetic Modifications
Heather Chapman, Eric M George, Univ of MS Medical Ctr, Jackson, MS

Preeclampsia is a common and serious obstetrical complication, hallmarked by new-onset hypertension and maternal endothelial dysfunction. Believed to result as a consequence of placental insufficiency and chronic placental ischemia, the symptoms of preeclampsia are caused by release of pathogenic factors from the placenta itself. A number of these factors have been identified, and it is likely that there are molecular mechanisms which remain obscure. The molecular regulation of even the known factors is often unclear. Here, we have used an established rodent model of placental ischemia which mimics many of the aspects of the human disorder to determine the direct effects of ischemia on global epigenetic modification in the placenta proper. In response to placental insufficiency, maternal blood pressure and rate of fetal demise increased significantly, while placental and fetal mass were decreased. Whole placental levels of histone H3 acetylation at K9 and K27 were increased ~2 and 3 fold respectively. Global methylation of cytosine from placental DNA was low in both groups (<1%), but there was ~50% increase in 5-mC in response to chronic ischemia. These results suggest that chronic placental ischemia induces global changes in epigenetic modification of chromatin in the placenta. This could be an important factor in the regulation of known and unknown pathogenic factors produced by the placenta in the preeclampsia patient.

H. Chapman: None. E.M. George: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; PI, NIH R00 Grant.

Funding: No

Proliferation of Endogenous T-regs Improves the Pathophysiology Associated with Placental Ischemia of Pregnancy

Tarek Ibrahim, Univ of MS Medical Ctr, Jackson, MS; Lukasz Przybyl, Experimental and Clinical Res Ctr, HELIOS Clinic, Berlin, Germany; Ashlyn C. Harmon, Denise C Connelius, Mark W. Cunningham Jr., Lorena M. Amaral, Jessica L. Faulkner, Univ of MS Medical Ctr, Jackson, MS; Ralf Dechend, Experimental and Clinical Res Ctr, HELIOS Clinic, Berlin, Germany; Babbette LaMarca, Univ of MS Medical Ctr, Jackson, MS

Preeclampsia (PE), new onset hypertension during pregnancy, is associated with pro-inflammatory cytokines and decreased regulatory immune mechanisms such as Tregs, IL-10 and IL-4. We believe this decrease in immune regulatory mechanisms leads to an uncontrolled proinflammatory response which contributes to most of the pathophysiology associated with PE. The Reduced Uterine Perfusion Pressure, RUPP, rat model of induced placental ischemia exhibits similar characteristics as women with PE including high blood pressure, elevated pro-inflammatory cytokines and cells and decreased Tregs, IL-4 and IL-10. Therefore, we hypothesized that stimulating Tregs by administration of a superagonist (SA) would increase the Treg profile in the RUPP rats which could reduce pro-inflammatory cytokines and blood pressure. The RUPP procedure was performed at gestation day 14 (GD14); SA was administered intraperitoneally at GD15, GD18 carotid catheters inserted, and GD19 MAP and pup weight, serum and tissues were collected. MAP in NP rats was 98.6 mmHg ± 4.71, 122.2 mmHg ± 1.84 in RUPPs which was improved to 110.789 mmHg ± 1.23 in RUPP+SA. Circulating FoxP3+ Treg cells were 5.987% ± 1.69% of total T-cells population in NP, 0.77% ± 0.49% in RUPP
rings but increased to 11.218% ± 2.9% in RUPP+SA; Circulating IL-6 levels were 41.008 ± 4.79 pg/mL in NP, 108.26 ± 25.99 pg/mL in RUPP, and 40.37 ± 4.49 pg/mL in RUPP+SA. Administration of the SA to the NP rats did not affect IL-6 Levels in comparison to NP at 36.79 ± 3.9 pg/mL. Plasma IL-10 Levels were at 58.399 ± 9.527 pg/mL in NP rats, these levels were significantly decreased in the RUPP rats, 26.298 ± 4.33 pg/mL, and treatment of the RUPPs with the SA significantly increased plasma IL-10 levels to 51.5486 ± 3.329 pg/mL. When the SA was given to NP rats, the levels for IL-10 were significantly higher compared to any of the other groups at 85.5207 ± 9.067 pg/mL. Placental Pre-pro Endothelin-1 (PPET-1) was increased 44.42 ± 0.269 fold in RUPP compared to NP 1 ± 0.255, but was decreased to 18.78 ± 0.48 in RUPP+SA. These data suggest an important role for up-regulating Treg cells to enhance the immune regulatory interactions and lower the hypertension without causing further reduction in fetal weight in response to placental ischemia during pregnancy.


Funding: No

Funding Component: P321

**Differential Leptin Levels are Associated with Hypertensive Disorders of Pregnancy and Adverse Pregnancy Outcomes**

**Donna A Santillan**, Whitney L Cowman, Sabrina M Scroggins, Mark K Santillan, Univ of Iowa, Carver Coll of Med, Iowa City, IA

During pregnancy, there are normal changes in maternal body weight and blood pressure that occur. Leptin is found in higher levels in obese individuals and has been demonstrated to have an inhibitory effect on myometrial contractility and to regulate blood pressure. Obesity in pregnancy is common and is associated with many complications, including hypertension in pregnancy, failed induction of labor (IOL), and intrauterine growth restriction. Our goal was to determine whether maternal leptin in a pregnant population is indicative of hypertensive disorders in pregnancy, dysfunctional labor, and whether there is a correlation between cord blood leptin and birthweight. We utilized a case control study with samples from the UI Maternal Fetal Tissue Bank (IRB#200910784). In order to analyze labor outcomes, 168 women were selected based on having undergone an IOL, including 54 failed IOL. Maternal/neonatal characteristics were collected from the medical record. Maternal and cord blood plasma leptin and total protein levels were measured using commercially available ELISAs. Bivariate analyses and logistic regression models were constructed using regression identified clinically-significant confounding variables. All variables were tested at significance level of 0.05. Women with hypertensive disorders in pregnancy, including pregnancy-induced hypertension and preeclampsia, had higher maternal leptin levels (14783 vs. 21440 pg/mL, p=0.049). Women with failed IOL also had higher maternal plasma leptin values (0.5 vs 0.3 leptin/protein [pg/ug], P = 0.01). Birthweight was also correlated with cord blood leptin (correlation coefficient 0.494 P < 0.001). These data suggest that maternal and fetal leptin levels are associated with the central mechanisms responsible for poor pregnancy outcomes.

**D.A. Santillan**: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA. F. Ownership Interest (includes any stock, stock option,
partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Patent on Diagnostics and Therapeutics in Preeclampsia. **W.L. Cowman:** None. **S.M. Scroggins:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA Postdoc Grant. **M.K. Santillan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Patent on Diagnostics and Therapeutics in Preeclampsia.

**Funding:** Yes  
**Funding Component:** Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

**P322**

**Vasopressin Release is Enhanced Before the Development of Preeclampsia in Humans Despite Exaggerated Suppression of Plasma Osmolality**

**Mark K Santillan,** Sabrina M Scroggins, Alyssa T Ray, Phillip C Witcher, Jeremy A Sandgren, Danny W Linggongorogo, Gary L Pierce, Donna A Santillan, Justin L Grobe, Univ of Iowa, Iowa City, IA

Plasma osmolality (Osm) suppression is of critical importance to maintain appropriate blood volume to perfuse the uterus during pregnancy. Osm is reduced starting at the fifth week of gestation via increased arginine vasopressin (AVP) secretion. This increased secretion is maintained via a decrease in the AVP/osmotic release threshold. We previously demonstrated that pregnant women who develop preeclampsia (PreE) exhibit exaggerated AVP secretion as early as the 6th week of gestation via measurement of copeptin, the stable C-terminal fragment of AVP. It is unclear whether AVP secretion is elevated before the onset of PreE due to osmotic or non-osmotic stimuli. We tested the hypothesis that elevated AVP secretion before PreE may be associated with elevated Osm (a strong stimulant of AVP secretion). Plasma and clinical data from pregnant women were obtained from the University of Iowa Maternal-Fetal Tissue Bank (IRB#200910784). Osm was measured using the freezing-point suppression technique. Osm was assessed in non-pregnant women (n=109), pregnant women who later developed PreE (n=12 for 7-12 weeks, n=9 for 16-24 weeks), and maternal and gestational age matched controls (n=25 for 6-13 weeks, n=15 for 14-27 weeks). As expected, Osm was decreased in control pregnancies (non-pregnant 291±1 vs pregnant 286±1 mOsm/kg, p<0.05). Contrary to our hypothesis, the Osm decrease was exaggerated in women who would later develop PreE (1st trimester: PreE 279±4 vs control 287±3, and 2nd trimester: PreE 277±4 vs control 285±3 mOsm/kg; effect of PreE p<0.05, gestational age p=NS, interaction p=NS) even after controlling for age, BMI, diabetes, chronic hypertension, history of preeclampsia, and gravida (model p<0.05). Despite suppressed Osm, plasma copeptin was elevated in the PreE group at all timepoints (p<0.05). These data support the conclusion that long before the development of clinical symptoms of PreE, the rate of secretion of AVP is inappropriately increased despite maintenance of normal osmotic-regulating actions of AVP. This effect must be the result of increased non-osmotic stimuli for AVP, and a suppression of the
AVP/osmotic release threshold beyond that observed in control pregnancies.

M.K. Santillan: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. S.M. Scroggins: None. A.T. Ray: None. P.C. Witcher: None. J.A. Sandgren: None. D.W. Linggonegoro: None. G.L. Pierce: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. D.A. Santillan: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. J.L. Grobe: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA.

Funding: Yes
Funding Component: National Center
P323

Arginine Vasopressin and Indoleamine 2,3 Dioxygenase: The Early Immunovascular Interface in Preeclampsia

Donna A Santillan, Sabrina M Scroggins, Eric J Devor, Stephen K Hunter, Justin L Grobe, Curt D Sigmund, Mark K Santillan, Univ of Iowa, Carver Coll of Med, Iowa City, IA

Preeclampsia (PreE), a hypertensive disease of pregnancy, causes maternal and fetal health complications. Abnormal placental development/angiogenesis and poor immunoregulation (involving T cells, Indoleamine 2, 3 Dioxygenase (IDO), and dendritic cells) are central to the development of PreE. IDO, produced by regulatory immune cells, degrades tryptophan to arrest inflammatory T cell proliferation and induces T regulatory cell development. Our data and others show decreased IDO placental expression from human preeclamptic pregnancies. We published that deletion of IDO (IDO KO) in pregnant mice results in pathognomonic glomerular endotheliosis, proteinuria, and intrauterine growth restriction, hallmark features of PreE. Our group also demonstrated that, plasma copeptin, a stable bio-marker of vasopressin (AVP) secretion, is elevated early in human PreE pregnancies; and AVP infusion into wild-type C57BL/6J dams phenocopies human PreE, including increased inflammatory T cells and highly activated dendritic cells. Here, we test our hypothesis that AVP (via copeptin measurement) is elevated in the IDO KO mouse model of PreE and that IDO activity is decreased in the AVP mouse model of PreE. Copeptin, measured by ELISA, was elevated in both the placenta (2.4±0.2 vs. 1.7±0.2 pg/mg, p=0.03) and maternal serum (2.1±0.2 vs. 1.2±0.4 pg/mg, p=0.03) from IDO KO pregnancies compared to wild-type at gestational day (GD) 18. In our chronic infusion of AVP model of PreE (24 ng/hour), GD 18 colorimetric IDO activity was decreased by 22% in the maternal kidney (N=10 per group) and by 27% in the amniotic fluid (saline N=8 vs. AVP N=12) of AVP-infused dams in comparison to controls. Collectively, these data demonstrate an inverse relationship between IDO activity and copeptin expression in PreE pregnancies. As both IDO and AVP sit at the crossroads between vascular and immune dysfunction, these data suggest that the IDO-AVP interaction may contribute to the maternal and fetal renal phenotype observed in preeclampsia.

D.A. Santillan: F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectual;
Reduction in Excreted Sodium Load in Obese Sheep: Role of Antenatal Betamethasone and Sex

Jianli Bi, Yixin Su, Mark Chappell, James Rose, Wake Forest Univ Sch of Med, Winston Salem, NC

Our previous studies have demonstrated the programmed impairment of sodium excretion in lean antenatal betamethasone exposed sheep whereby the males exhibited blunted excretion of a sodium load when compared to control males or control or antenatal betamethasone exposed females. Obesity has been recognized as a risk factor for developing early stage kidney failure in individuals exposed to unfavorable intrauterine conditions by virtue of fetal programming. We hypothesize that obesity (second hit) can accentuate the impairment of sodium excretion in antenatal betamethasone exposed offspring in a sex specific manner. Pregnant ewes received either betamethasone or vehicle at 80-81 days gestation. The male (n=6 per group) or female (n=5 per group) offspring were overfed to become obese (40% weight gain over a course of 3 months) at age of 1.5 years. Acute sodium load (sodium chloride: 0.13g/kg) was administrated intravenously. Sodium excretion (excreted sodium load), blood pressure (BP), renal plasma flow (RPF), and glomerular filtration rate (GFR) were measured. Data for sodium excretion are expressed as percentage of excreted sodium load. ANOVA and Student’s t test were used for data analysis. The excreted sodium load was lower in obese male antenatal betamethasone exposed offspring (OMB) than in obese male vehicle exposed offspring (OMV) (13.5±3.1% vs. 33.2±9.9% p=0.04). However, the excreted sodium load was not significantly different between obese female antenatal betamethasone exposed offspring (OFB) and obese female vehicle exposed offspring (OFV) (60.4±6.2% vs. 72.0±8.2%, p=0.31). Compared male offspring with female offspring, the excreted sodium load was lower in male offspring (33.2±9.9% vs. 72.0±8.2%, p=0.001, OMV vs. OFV; 13.5±3.2% vs. 60.4±6.2%, p<0.0001, OMB vs. OFB). During the experiments BP, RPF and GFR did not change significantly.

These data suggest that obesity, acting as a second hit, accentuates impairment of sodium excretion in antenatal betamethasone exposed male but not female offspring. Thus the sex specific effect of antenatal betamethasone on
sodium excretion persists in animals experiencing a secondary insult of obesity in adulthood.

J. Bi: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH Grant HD-47584, NIH Grant HD-17644. Y. Su: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH Grant HD-47584, NIH Grant HD-17644. M. Chappell: None. J. Rose: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH Grant HD-47584, NIH Grant HD-17644.

Funding: No Funding Component: P325

Enhanced Afferent Arteriole Dilatation in Dahl Salt-sensitive Rats (dahlss): Role of Connecting Tubule Glomerular Feedback (ctgf)

Hong Wang, Cesar A Romero, Branislava Janic, Edwards Peterson, Oscar A Carretero, Henry Ford Hosp, Detroit, MI

Afferent arteriole (Af-Art) resistance is modulated by 2 intrinsic nephron feedbacks: the vasoconstrictor tubuloglomerular feedback (TGF) and the vasodilator CTGF. TGF is mediated by NKCC2 channel in the macula densa and blocked by furosemide; and CTGF is mediated by ENaC in the connecting tubule and blocked by benzamil. Previously we measured CTGF indirectly, by differences between TGF response with and without CTGF blocker benzamil. Thus, using this indirect measurement we reported that Dahl SS have greater CTGF than Dahl salt-resistant rats (Dahl SR). We have recently developed a new method to measure CTGF more directly and we found that when we simultaneously blocked TGF with furosemide and CTGF with benzamil, the increasing tubular perfusion caused Af-Art constriction (TGF-like) that is mediated by the NHE. We hypothesize that in vivo during simultaneous inhibition of NKCC2 and the NHE, CTGF causes an Af-Art dilatation revealed by an increase in stop-flow pressure (Psf) and that is greater in Dahl SS than in Dahl SR in a high salt diet. In the presence of furosemide alone, increasing nephron perfusion did not change the Psf in neither Dahl SS nor Dahl SR. When we blocked both, NKCC2 with furosemide and NHE with DMA, increase in tubular flow caused Af-Art dilation that was demonstrated by an increase in Psf. This increase was greater in Dahl SS (5.1±0.4 mmHg) than in Dahl SR (2.9±0.3 mmHg; P < 0.01), (Fig).We confirm that CTGF causes this vasodilation, since benzamil completely blocked this effect. We conclude that during inhibition of NKCC2 and NHE in the nephron CTGF (Af-Art dilatation) is enhanced in Dahl SS as compared to Dahl SR.


Funding: No Funding Component: P327
Increased Brain iNOS Contributes to Hypertension in Dahl Salt Sensitive Rats

Michael J Huber, Fengli Zhu, Robert A Larson, Qing-Hui Chen, Zhiying Shan, Michigan Technological Univ, Houghton, MI

The hypothalamic paraventricular nucleus (PVN) is one of the key central nuclei to play an important role in regulating arterial blood pressure (ABP) of salt-sensitive hypertension (SSH). However, the detailed molecular mechanism(s) whereby the PVN increases ABP are not well understood. Here, we tested the hypothesis that high salt (HS) loading increases expression of iNOS in the PVN which contributes to SSH. Six-week-old male Dahl salt sensitive (Dahl S) rats and age matched Sprague Dawley (SD) rats were fed either a HS (4% NaCl) or a normal salt (NS, 0.4% NaCl) diet (n=4~7/group). Mean arterial pressure (MAP) was measured via tail cuff method. Five weeks following diet treatment, HS diet induced hypertension in Dahl S rats (HS: 153±9; vs. NS: 122±2 mmHg, P<0.05), but not in SD rats (HS: 107±3; vs. NS: 107±2 mmHg). Rats were then euthanized and PVN tissues were punched out for real time PCR. The HS diet induced dramatic increases in mRNA levels of iNOS (25-fold), and Fra1 (3.6-fold), a chronic neuronal activation marker, in Dahl S rat but not in SD rats. Next, we investigated the effect of intracerebroventricular (ICV) administration of hypertonic saline on PVN iNOS and Fra1 expression in SD rats. Anesthetized adult male SD rats received ICV infusion of isotonic NaCl (0.15 M, 2µl, as control) or hypertonic NaCl (2M, 2µl) (n=7~8/group). Three hours following ICV infusion, rats were euthanized and PVN mRNA levels of iNOS and Fra1 were assayed. ICV hypertonic saline increased mRNA levels of iNOS (9.5-fold) and Fra1 (4.1-fold). We further tested whether these increases in iNOS and Fra1 expression occurred in neurons. Incubation of hypertonic saline (10 mM NaCl) for 3 hours increased iNOS (6-fold) and Fra1 (2.8-fold) mRNA levels in neuronal cultures from the hypothalamus containing the PVN. Finally, we tested whether increased iNOS activity contributes to ABP elevation in Dahl SSH. In anaesthetized Dahl S rats, bilateral PVN microinjection of the iNOS inhibitor, aminoguanidine (250 pmol) significantly decreased MAP in HS treated animals compared to rats with a NS diet (HS: -13±3; vs. NS: -2±2 mmHg, P<0.05) (n=5/group). These observations suggest that HS intake increases iNOS expression in PVN neurons, which may contribute to the central neural mechanism of Dahl SSH.

M.J. Huber: None. F. Zhu: None. R.A. Larson: None. Q. Chen: None. Z. Shan: None.

Funding: Yes
Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

Reduced Salt Taste Sensitivity Leads to Increase of Daily Salt Intake

Minoru Isomura, Toru Nabika, Shimane Univ, Izumo, Japan

Objective: It is empirically aware that inter-individual differences exist in sensitivity of salt taste. It is assumed that person with reduced sensitivity to salt taste may consume a higher amount of salt than those with a normal sensitivity of salt. The objective of this study is to reveal the relationship between the sensitivity of salt taste and salt intake. Design and method: In this study, we defined salt taste sensitivity by the threshold of the sense of salt taste. Therefore, we defined as reduced sensitivity when individuals were unable to
sense salt taste in the concentration that majority of individuals could. Japanese residents who came to annual health checkups were recruited to this study. A series of salt-impregnated taste strips were used to measure salt taste intensity. Each taste strip was impregnated with sodium chloride at concentrations of 0, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 g/cm². Daily sodium intake was estimated from sodium excretion in the spot urine using Tanaka’s equation. Results: Among 1,207 participants, 979 (81.1%) individuals were considered to bear normal sensitivity because they were able to detect salt taste by taste strip of 0.6 or 0.8 g/cm². However, 67 (5.6%) individuals were categorized as reduced sensitivity group because they were able to detect it only by the taste strip with the highest amount of salt, or unable to detect salt taste. Daily salt intake of reduced sensitivity group was significantly higher than those in normal sensitivity group (10.3g vs 9.7g, p<0.04). No significant difference was identified in blood presser. Salt taste sensitivity decreased by age, history of smoking and presence of diabetes mellitus.

Conclusions: Our study revealed the sensitivity of salt taste is associated with daily salt intake, even adjusted by gender and age. Strict reduction of salt is recommended especially for Smokers or persons with diabetes mellitus, not only because low BP recommendation but also they might pretend to consume salt because of reduced sensitivity of salt taste.

M. Isomura: None. T. Nabika: None.

Funding: No

Funding Component: P329

Age- and Sex-differences in Salt Sensitivity and Renal Function in a Rat Model of Autosomal Recessive Polycystic Kidney Disease

Chunhua Jin, Michal Mrug, Bradley K. Yoder, Robert A. Kesterson, Edward W. Inscho, David M. Pollock, Univ of Alabama at Birmingham, Birmingham, AL

Background:
Most forms of hypertension are influenced by gender and salt sensitivity, however, the interplay of these two factors and their functional consequences in polycystic kidney disease (PKD)-associated hypertension are poorly understood. Thus, we tested the hypothesis that age and male sex would predispose animals with PKD to hypertension and risk of renal dysfunction.

Methods:
We used the Pkhd1pck rat model of autosomal recessive PKD to study sex differences in blood pressure in response to a high salt diet (HSD). Two month- and eight month-old male and female Pkhd1pck rats were surgically implanted with telemetry transmitters and allowed to recover for at least one week before obtaining baseline mean arterial pressure (MAP). Rats were maintained on either HSD (4% NaCl) or normal rat chow (0.49% NaCl) for 3 weeks. At the end of the study, rats were placed in metabolic cages and a 24 hr urine sample was collected before measuring GFR (transdermal sinistrin clearance).

Results:
In the 2-month old Pkhd1pck rats, blood pressures were in a normal range and there were no differences between males and females. Furthermore, 3 weeks on a HSD had no effect on 24 hr MAP. GFR was similar between male and female rats on either diet. However, urinary protein excretion was significantly higher in HSD fed rats compared to normal rat chow in both male (83±26 vs. 17±4 mg/day, respectively, p<0.05) and female rats (106±25 vs. 6±2 mg/day, p<0.05). In 8-month old rats, again there were no sex differences between animals on normal rat chow. However, MAP
increased progressively in male rats after 3 weeks of HSD feeding, a change that was significantly greater than females (Δ39±6 vs Δ15±5 mmHg, p<0.05). The blood pressure increase in male rats was associated with higher urinary protein excretion compared to female rats (755±118 vs. 390±48 mg/day, p<0.05). HSD significantly reduced GFR in male, but not female rats (0.48±0.13 vs. 1.24±0.05 mL/min/100 g bwt, respectively, p<0.05). GFR was similar between older male and female rats on normal salt diet.

Summary:
Our studies demonstrate that male Pkhd1pck rats with advanced cystic kidney disease are more vulnerable to salt sensitive hypertension and renal injury than age-matched females.


Funding: No
Funding Component: P330

Role of Na-H Exchanger Regulatory Factor 1 (NHERF1) In Salt-sensitive Hypertension and Inflammation in Aging

Syed J Khundmiri, Howard Univ, Washington, DC

Aging animals develop salt-sensitive hypertension due, in part, to desensitization of dopamine receptors and sensitization of AT1R in renal proximal tubules. We demonstrated old FBN rats develop salt-dependent hypertension. Preliminary data showed loss of NHERF1 expression in 22m old F344 rats. We hypothesized that loss of NHERF1 may contribute to salt reabsorption in aging. To address this hypothesis, Fischer Brown Norway (FBN) rats (1m, 4m, 12m, and 24m old) were fed 1% or 8% NaCl diet for one week and, urinary volume, sodium, potassium, and chloride and expression of Na-K ATPase α1 subunit (NKA), NHERF1, and D1R were measured. Feeding 8% NaCl increased 24 h urine volume compared to rats fed 1% NaCl diet in 1m (13.6±0.98 vs 29.2±3.8 mL) and 4m (15.3±1.5 vs 39.2±2.4) but not in 24 m rats (20.7±4.7 vs 15.9±2.4). 24 h urine Na increased 7 fold in 4m old rats fed an 8% NaCl diet but only 3 fold in 24m old rats. To our surprise, NHERF1 expression increased with age in FBN rats (0.21±.03 (4m) vs 0.71±0.07 (24m) AU ratio NHERF1:actin). In F344 rats NHERF1 expression decreased with age (0.86±0.14 vs 0.39±0.05). Increased NHERF1 expression with age in FBN rats was associated with decreased D1R expression (0.8±0.16 vs 0.4±0.04) while in F344 rats D1R expression increased with age (0.6±0.1 vs 0.9±0.1). FBN rats are salt-sensitive while F344 are salt-resistant. F344 rats develop kidney inflammation with aging. We determined TNFα in kidney homogenates from FBN and F344 rats. TNFα increased with age in both F344 (4.04±0.15 vs 6.9±0.42) and FBN rats (2.1±0.3 vs 4.05±0.31 ng/mg protein). To determine if lack of NHERF1 is responsible for salt wasting, we measured urine volume and Na in 18 m old WT and NHERF1 KO mice. The urine volume in WT mice (2.7±0.8 (1% NaCl) vs 5.6±1.2 mL/24 h (8% NaCl)) were significantly lower than KO mice (3.6±0.57(1% NaCl) vs 7.5±0.88 mL/24 h (8% NaCl)). Similar results were observed in Na excretion. D1R expression increased in NHERF1 KO mice as compared to WT mice. TNFα increased significantly in NHERF1 KO mice kidneys as compared to WT mice. We conclude that increased NHERF1 expression as seen in FBN rats results in salt retention while lack of NHERF1 as seen F344 rats and NHERF1 KO mice increases renal inflammation. Further studies are required to delineate the two roles of NHERF1.

S.J. Khundmiri: A. Employment; Significant; Howard University. B. Research Grant (includes principal investigator, collaborator, or
consultant and pending grants as well as grants already received); Significant; NIH 7R21AG047474.

Funding: No
Funding Component: P331

**Renal Medullary Infusion of BAF312, a Sphingosine-1-phosphate Receptor-1 Agonist, Attenuates Angiotensin II-induced Hypertension**

Qing Zhu, Junping Hu, Pin-Lan Li, Ningjun Li, Medical Coll of Virginia, Virginia Commonwealth Univ, Richmond, VA

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite formed by phosphorylation of sphingosine and participates in the regulation of cardiovascular functions. We have recently shown that S1P increases sodium excretion in the renal medulla possibly through inhibiting epithelial sodium channel via the S1P receptor 1 (S1P1), which is mainly localized in collecting ducts with a higher expression level in the renal medulla than the cortex. The present study tested the hypothesis that infusion of an agonist to activate S1P1 in the renal medulla attenuates angiotensin (ANG) II-induced hypertension. Treatment of the mice with a high salt diet (HS, 4% NaCl) for 2 week significantly increased the levels of S1P1 in the renal medulla compared with that in low salt (LS) control by Western blot analysis, whereas this HS-induced increase in S1P1 level was blocked in mice treated with ANG II (600ng/kg/min, sc) (relative S1P1 levels: 1.0±0.19, 1.9±0.14 and 0.9 ±0.18 in LS, HS and HS+ANG II-treated mice, respectively). Infusion of a subpressor dose of ANG II (300ng/kg/min, sc) increased the mean arterial pressure (MAP) in mice with collecting duct-specific knockout of S1P1, but not in S1P1 floxed control mice (MAP: 132±4.7 vs. 96±1.1 mmHg). In contrast, infusion of BAF312, a selective agonist of S1P1, into the renal medulla attenuated the hypertension induced by a pressor dose of ANG II (600 ng/kg/min, sc) in uninephrectomized mice (MAP: 102±1.8, 161 ±7.1 and 133±3.9 in vehicle, ANG II and ANG II+BAF312-treated mice, respectively). These data suggest that inhibition of S1P1 level in the renal medulla may contribute to the pathogenesis of ANG II-induced hypertension and that stimulating the S1P1 pathway may be used as a therapeutic strategy for the treatment of hypertension.

Q. Zhu: None. J. Hu: None. P. Li: None. N. Li: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, HL089563.

Funding: No
Funding Component: P332

**Role of T-Lymphocytes in the Long-Term Blood Pressure and Renal Disease Effects of Acute Renal Ischemia-Reperfusion Injury**

Bernardo Lopez, Galina Petrova, Justine M Abais-Battad, Hayley Lund, Daniel Fehrenbach, David L Mattson, Medical Coll of Wisconsin, Milwaukee, WI

Epidemiological data indicates that acute kidney injury (AKI) is an independent risk factor for the development of hypertension and chronic kidney disease in patients. Previous studies demonstrated that rats develop sodium-dependent hypertension and kidney damage following experimental AKI induced by a renal ischemia-reperfusion (IR) insult; furthermore, these high salt deleterious effects could be blunted by administration of immunosuppressive agents. The present study was performed on Dahl SS (SS) rats and SS rats with a null mutation in the CD247 gene (SS-CD247) leading to depletion of T-lymphocytes in order to specifically examine the role of T cells...
in this response (n=5-6 rats/group). As assessed by serum creatinine (Scr) levels, no difference was observed in the initial response to IR injury between SS and SS-CD247: Scr increased from 0.44±0.03 to 2.16±0.32 mg/dl in SS rats 24 hours after an initial 30 minute period of renal ischemia and returned to control levels after 8 days of recovery. Moreover, no differences were noted in mean arterial pressure (MAP) or albumin excretion rate (UAib) between SS and SS-CD247 after 43 days of recovery from IR injury while the rats were maintained on a low salt (0.4% NaCl) diet. When the rats were fed a 4.0% NaCl diet for two weeks, MAP and UAib significantly increased in the sham SS to 178±9 mmHg and 87±17 mg/day, respectively; values significantly greater than observed in the sham SS-CD247 rats (148±2 mmHg and 87±17 mg/day). As expected, the SS rats recovered from IR injury demonstrated an exaggerated increase in MAP (peaking at 183±2 mmHg) and UAib (275±54 mg/day) in response to high salt. There was no difference in the number of total CD3+ lymphocytes in the kidneys of IR and sham SS after high salt, though the ratio of CD4+/CD8+ T cells was increased in the IR group. Compared to sham CD247, an exaggerated elevation of MAP (157±9 mmHg) and UAib (210±32 mg/day) was also observed in the SS-CD247 rats recovered from IR injury, demonstrating enhanced responsiveness following IR injury in animals lacking T cells. These data indicate that T lymphocytes amplify salt-sensitive hypertension and renal damage, but other mechanisms also mediate the salt-sensitive hypertension and renal damage that occurs in animals recovered from IR injury.

B. Lopez: None. G. Petrova: None. J.M. Abais-Battad: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH and AHA Grants.

Funding: No
Funding Component: National Center P333

**The Impact of Sodium on Blood Pressure and Arterial Stiffness is Moderated by Indices of Obesity in People of African Ancestry.**

Maseko Muzi, Univ of the Witwatersrand, Johannesburg, South Africa

**Background:** Obesity is on the rise worldwide and like Na⁺ it is associated with blood pressure (BP) and target organ changes. Our study population has a high incidence of obesity (67%) and a dietary sodium intake that is slightly above the recommended threshold. Previous studies conducted in this population have shown no relationship between Na⁺ and both BP and arterial stiffness. With the high incidence of obesity in this population, it is possible that the indices of obesity blunt this relationship. Therefore in this study we investigate whether the relationship between Na⁺ and both BP and PWV is moderated by the indices of obesity. **Methods:** We recruited 1219 South Africans of African ancestry and measure 24-h ambulatory on 796 participants and 597 had complete 24-hour urine collection. Anthropometric measurements were taken and a standard questionnaire was issued to determine lifestyle habits and history of medication. To assess arterial stiffness we used applanation tonometry to measure pulse wave velocity (PWV). **Results:** After correcting for covariates, there was an association between Na⁺ and PB in participants with normal BMI but not in obese participants. Similarly there was a relationship between Na⁺ and PWV (p=0.0447) in individuals with normal BMI only. When waist circumference was used as an index of obesity,
gender disparities were observed in the relationship between Na\(^+\) and PWV. There was no relationship between Na\(^+\) and PWV irrespective the waist circumference in males but a multivariate regression analyses showed a relationship between Na\(^+\) and PWV in all women (p=0.0142) and in women with a normal waist circumference (p=0.0006). **Conclusion:** In a population with a high incidence of obesity, the relationship between Na\(^+\) and both BP and PWV is modified by the indices of obesity.

M. Muzi: None.

Funding: No

Funding Component: P334

**Sympathetically Mediated Alpha-1 Adrenoceptor Regulation of the NCC During High Salt Intake: A New Therapeutic Target for Resistant Hypertension**

Richard D Wainford, Kathryn R Walsh, Boston Univ, Boston, MA

**Aim:** We hypothesize that excess norepinephrine (NE) modulates NCC activity via an α1 adrenoceptor pathway to drive the development of salt-sensitive hypertension (HTN).

**Methods:** Male Sprague-Dawley (SD) rats receiving a continuous s.c. saline or NE (600ng/min) infusion and naïve Dahl Salt-Sensitive (DSS) rats were fed a 0.6% (NS) or 8% NaCl (HS) diet for 14 or 21 days respectively (N=4/gp). On day 14 (SD) or 21 (DSS) MAP and NCC activity (peak natriuresis to iv hydrochlorothiazide (HCTZ; 2mg/kg) infusion) and expression (via immunoblotting) was assessed. Additional groups of NE infused SD and DSS rats received a propranolol (9.9mg/kg/day; s.c.) or prazosin (2.5mg/kg/day; oral) and a NS or HS diet for 14 or 21 days.

**Results:** SD rats exhibit HS evoked suppression of NCC expression and activity. In contrast, NE infused SD rats and DSS rats exhibit HTN and fail to suppress NCC expression and activity during HS-intake. β-adrenoceptor antagonism (confirmed pharmacologically) reduced MAP in NE infused SD and DSS rats, but failed to decrease NCC activity or expression. In contrast α1-adrenoceptor antagonism (confirmed pharmacologically) abolished the salt-sensitive component of HTN and restored dietary sodium evoked suppression of NCC activity and expression in NE infused SD rats and DSS rats.

**Conclusion:** Our data suggests NE activates α, but not β, adrenoceptors to prevent dietary sodium evoked suppression of NCC activity and the development of salt-sensitive hypertension. The PATHWAY-2 Trial reported a primary role of sodium retention in resistant HTN suggesting α1-adrenoceptor antagonism represents a new therapeutic approach for resistant and sympathetically mediated HTN.

<table>
<thead>
<tr>
<th>Dietary salt intake</th>
<th>MAP (mmHg)</th>
<th>Peak natriuresis to HCTZ (mg/kg/hr)</th>
<th>NCC expression (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD inf</td>
<td>HS (5%) NaCl</td>
<td>124±2</td>
<td>6.7±0.3</td>
</tr>
<tr>
<td>DSS inf</td>
<td>HS (5%)</td>
<td>124±2</td>
<td>7.2±0.4*</td>
</tr>
<tr>
<td>SD inf</td>
<td>HS (8%) NaCl</td>
<td>171±2</td>
<td>11.5±2.1</td>
</tr>
<tr>
<td>DSS inf</td>
<td>HS (8%)</td>
<td>171±2</td>
<td>10.5±1.4</td>
</tr>
<tr>
<td>SD inf</td>
<td>NE (600 ng/min) inf</td>
<td>130±5</td>
<td>10.7±1.2</td>
</tr>
<tr>
<td>DSS inf</td>
<td>NE (600 ng/min)</td>
<td>130±5</td>
<td>9.6±1.2</td>
</tr>
<tr>
<td>SD inf</td>
<td>NE (600 ng/min) + Propranolol</td>
<td>130±5</td>
<td>8.4±1.2</td>
</tr>
<tr>
<td>DSS inf</td>
<td>NE (600 ng/min) + Propranolol</td>
<td>130±5</td>
<td>9.2±1.2</td>
</tr>
<tr>
<td>SD inf</td>
<td>NE (600 ng/min) + Prazosin</td>
<td>130±5</td>
<td>10.7±1.2</td>
</tr>
<tr>
<td>DSS inf</td>
<td>NE (600 ng/min) + Prazosin</td>
<td>130±5</td>
<td>10.4±1.0</td>
</tr>
</tbody>
</table>

**Table 1:** *p<0.05 vs. respective NS group; **p<0.01 vs. SD or saline HS group; ***p<0.01 vs. DSS HS group. N.D. = not determined.

R.D. Wainford: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH RO1 HL107330, K02 HL112718, Novartis Investigator Initiated Support. D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Japanese Hypertension Summit 2016. E. Honoraria; Modest; Emory University
The Cross-talk Between Kidney and Retroperitoneal Adipose Tissue Reveal Novel Roles for Adiponectin in the Regulation of Sodium Excretion in Essential Hypertension

Chunyu Zeng, Hefei Huang, Daping Hosp, Chongqing, China

Rationale—Obesity related hypertensive patients are often with impaired sodium excretion. However, the mechanisms are not clear. Adiponectin is an important adipocytokine involved in the regulation of essential hypertension. We studied the role of adiponectin in the cross-talk between retroperitoneal adipose tissue and renal sodium excretion in physiological state and essential hypertension.

Objective—We tested if and how adiponectin enhances natriuresis and diuresis, and the impaired adiponectin-induced sodium excretion might be involved in essential hypertension.

Methods and Results—The retroperitoneal fat in spontaneously hypertensive rat (SHR) is increased, and with lower expression of adiponectin and increased Na⁺-K⁺-ATPase activity of renal proximal tubule cells (RPTCs). Infusion of adiponectin via supracapsular artery induces natriuresis and diuresis in Wistar-Kyoto rats (WKY). Treatment with adiponectin inhibited Na⁺-K⁺-ATPase activity in RPTCs, via adiponectin receptor 1(AdipoR1) and adiponectin receptor 2(AdipoR2), activated AMP-activated protein kinase (AMPK) - endothelial nitric oxide synthase(eNOS) signal way. However, In SHRs, adiponectin-induced natriuresis and diuresis, and the inhibitory effect on Na⁺-K⁺-ATPase activity were lost. The R₁ and R₂ expression in RPTCs in SHRs was lower, the AMPK-eNOS pathway were also impaired. Transfected with both R₁ and R₂ to RPTCs from SHR restored the inhibitory effect of adiponectin on Na⁺-K⁺-ATPase activity.

Conclusions—Adiponectin, involved in the regulation of cross-talk of retroperitoneal fat and kidney in hypertension, via R₁ and R₂, induces natriuresis and diuresis; the impaired adiponectin function and decreased expression of AdipoR might be involved in the pathogenesis of hypertension.

C. Zeng: None. H. Huang: None.

Funding: No

Funding Component: P335

Changes in Arterial Pulse Wave Velocity, Effective Arterial Elastance, and Contractility in Hypertension Induced Cardiovascular Disease in Rats: Independent Prognostic Values

Steve R Roof, Carlos del Rio, QTest Labs, Columbus, OH

Hypertension is an established comorbidity in cardiovascular (CV) disease. Changes in aortic pulse-wave velocity (PWV) have been shown to predict the longitudinal development of hypertension, its underlying vascular alterations, and associated CV risks. However, as the heart and arterial tree are coupled, it has been shown that the successful monitoring of not only CV disease progression, but of its therapies, requires the evaluation of ventriculo-arterial uncoupling and its determinants. This study aimed to establish the relationships between PWV and changes in the estimated effective arterial elastance (Ea) and/or contractility over a wide-range of hypertension-induced CV pathologies in a controlled rodent model/environment.

In a large series (n = 96) of anesthetized (pentobarbital) male Sprague-Dawley rats, PWV
(carotid-to-femoral), systolic pressures, contractility (preload recruitable stroke work; PRSW), and Ea were evaluated using echocardiography, invasive hemodynamics, and left ventricular (LV) pressure-volume relationships. Data were collected in healthy animals (n = 37) and those with hypertension-induced chronic systolic/diastolic left-ventricular dysfunction (n = 59) (chronic beta-adrenergic stimulation and/or renoprival). A subset of each group, were also studied under common clinical cardio/vasoactive pharmacological interventions.

Disease animals had significantly (P < 0.001) higher systolic pressures (141 ± 4 vs 116 ± 4 mmHg), faster PWV (9.0 ± 0.8 vs 5.1 ± 0.5 m/s), and depressed contractility (PRSW: 39 ± 1 vs. 49 ± 1 mmHg*). Both PWV (7.6 ± 0.7 to 4.3 ± 0.4 m/s) and Ea (95 ± 10 to 63 ± 8 mmHg/mL) decreased in response to vascular therapies in the diseased animals; only PWV was reduced in healthy rats. More interestingly, over all conditions, PWV was a poor predictor of both systolic pressures (R²=0.00009) and Ea (R²= 0.024). but yet was a moderate predictor of PRSW (R²=0.23, P < 0.001), suggesting a contractility-dependent velocity of propagation. Taken together, these data suggest that indices of ventriculo-arterial efficacy, cardiac afterload, and peripheral arterial stiffness while all are affected by CV disease, each cannot be used to predict one another, and all provide independent prognostic information in disease.

S.R. Roof: None. C. del Rio: None.

Funding: No
Funding Component: P337

Mustafa Lokhandwala, Andrea Diaz Diaz, Anees Ahamd Banday, Heart and Kidney Inst, Coll of Pharmacy, Univ of Houston, Houston, TX

The role of angiotensin in etiology of cardiovascular diseases especially in hypertension is well established. Renin-angiotensin-aldosterone contributes to the development and maintenance of hypertension directly by increases in vascular tone and renal sodium reabsorption or indirectly by increasing oxidative stress and inflammation. Contrary to this pathological arm, angiotensin (Ang) 1-7 via Mas receptors has been reported to protect the cardiovascular function although the exact mechanism is not yet clear. We have previously shown that oxidative stress leads to renal dopamine D1 receptor (D1R) dysfunction which could disrupt sodium regulation and subsequently lead to hypertension. In here we wanted to test whether chronic administration of Ang 1-7 in mice could mitigate oxidative stress, protect renal D1R function and prevent development of hypertension. Mice (C57BL) were implanted with telemetry probes and concomitantly treated with L-buthionine sulfoximine (BSO, in drinking water) and Ang 1-7 (via jugular vein by osmotic pumps). Control (C, no treatment) and shams (implanted with saline filled pumps) exhibited similar behavioral and physiological parameters. Mice treated with BSO alone exhibited increased oxidative stress and high BP as compared to controls. Ang 1-7 treatment did not affect oxidative stress and BP in control mice but prevented the increase in BP and oxidative milieu in BSO treated mice. Mean arterial pressure (mmHg), C: 78.5 ± 2.3*; BSO: 97.3 ± 3.8; Ang 1-7: 80.1* ± 4.1; BSO+Ang 1-7: 83.2 ± 3.4*, *P <0.05 vs BSO. SKF38393, a D1R agonist, increased urine and sodium excretion in control mice but failed to induce diuresis or natriuresis in BSO-treated mice. Treatment with Ang 1-7 protected D1R function as both natriuresis and diuresis was

Angiotensin 1-7 Mitigates Oxidative Stress, Protects Renal Dopamine D1 Receptor Function and Prevents Development of Hypertension
observed in mice treated with BSO plus Ang 1-7. Chronic Ang 1-7 had no effect on D1R function in the absence of BSO. These data show that oxidative stress leads to hypertension by disrupting renal D1R dependent sodium regulation. Ang 1-7 mitigates oxidative stress, protects renal D1R function and prevents increase in BP. This study provides a new insight on how beneficial arm of Ang system could protect renal D1R-mediated sodium regulation and prevent development of hypertension during oxidative stress.

M. Lokhandwala: None. A. Diaz Diaz: None. A. Banday: None.

Funding: No
Funding Component: P338

Heme Oxygenase Induction Suppresses Hepatic Hepcidin and Rescues Ferroportin and Ferritin Expression in Obese Mice

Hibba Chaudhry 25701, Alexandra Nichols, Komal Sodhi MD, Krithika Srikanthan MD, Athar Nawab, Marshall Univ Sch of Med, Huntington, WV

Hepcidin, a phase II reactant secreted by hepatocytes, regulates cellular iron levels by increasing internalization of ferroportin- a transmembrane protein facilitating egress of cellular iron. Chronic low-grade inflammatory states, such as obesity, have been shown to increase oxidative stress and enhance hepcidin secretion from hepatocytes and macrophages. Heme-heme oxygenase (HO) is a stress response system, the induction of which reduces oxidative stress thereby abating pathophysiological conditions such as obesity and metabolic syndrome. 8 week old male obese (ob) mice and their age- and sex-matched lean mice were used as controls. CoPP was administered intraperitoneally once a week (3 mg/kg) for 6 weeks. CoPP plus stannous mesoporphyrin (SnMP) was administered intraperitoneally three times a week (20 mg/kg) for 6 weeks.

We investigated the effects of HO-1 induction on hepatic hepcidin levels and on iron homeostasis in tissues from lean and obese mice. Obese mice exhibited hyperglycemia along with increased levels of pro-inflammatory cytokines (MCP-1, IL-6, p<0.05), oxidative stress and increased hepatic hepcidin levels (p<0.05). Enhancement of hepcidin was reflected in the reduced expression of ferroportin in obese mice (p<0.05). Further, our results showed attenuation of insulin receptor phosphorylation and attenuation of metabolic regulators including pAMPK, pAKT and pLKB1. Cobalt protoporphyrin (CoPP)-induced HO-1 up-regulation in obese mice and reversed these pathophysiological alterations (p<0.05) while attenuating hepatic hepcidin levels and enhancing ferritin expression. These effects of CoPP were prevented in obese mice concurrently exposed to an inhibitor of HO (SnMP) (p<0.05).

Taken together, our results highlight a modulatory effect of HO on iron homeostasis mediated through the suppression of hepatic hepcidin in conjunction with the rescue of cellular ferritin levels. Therefore, these findings may prove an effective strategy in treating the metabolic consequences of obesity including alteration of liver iron homeostasis.


Funding: No
Funding Component: P339

Endothelium-specific Deletion of Amyloid Precursor Protein Causes Endothelial Dysfunction of Cerebral Arteries

Livius V. d’Uscio, Zvonimir S. Katusic, Mayo Clinic, Rochester, MN
Amyloid-β precursor protein (APP) is an integral membrane protein expressed abundantly in the endothelium of cerebral arteries. However, the exact physiological function of APP in endothelial cells is unknown. Mice with vascular endothelium-specific deletion of APP gene (eAPP-KO) were generated using loxP/Cre technology to test the hypothesis that APP plays a key role in the control of vascular endothelial nitric oxide (NO) function. Vasoreactivity of isolated basilar arteries were studied in vitro under pressurized conditions and compared to control littermates. eAPP-KO mice were normotensive (118±2 mmHg vs. control mice: 113±2 mmHg; n=5) and plasma cholesterol and blood glucose levels were not affected by endothelium-specific genetic inactivation of APP. Endothelium-dependent relaxations to acetylcholine were significantly impaired in eAPP-KO mice (maximal relaxation: 30±4% vs. 45±5% for control littermates; P<0.05; n=7-8) while endothelium-independent relaxations to NO-donor diethylamine-NONOate were unchanged (n=5-6). Western blot analysis revealed that protein expression of endothelial nitric oxide synthase (eNOS) was significantly downregulated by 31% in cerebral arteries of eAPP-KO mice (P<0.05 vs. littermates; n=6). Furthermore, basal levels of cyclic guanosine monophosphate were also significantly reduced in cerebral arteries of eAPP-KO mice (0.64±0.09 pmol/mg; P<0.05 vs. littermates: 0.97±0.11 pmol/mg; n=11). In contrast, protein expression of prostacyclin synthase as well as levels of cyclic adenosine monophosphate were not affected by genetic inactivation of APP in endothelial cells (n=5-7). Moreover, superoxide anion levels as determined by HPLC analysis of 2-hydroxyethidium were unaltered in eAPP-KO mice cerebral arteries (1.4±0.2 nmol/mg vs. littermates: 1.2±0.1 nmol/mg; P=n.s.; n=7). Our results demonstrate that impaired endothelium-dependent relaxations to acetylcholine in eAPP-KO mice are caused by the reduced expression and function of eNOS. These findings indicate that under physiological conditions APP expression in cerebral vascular endothelium plays an important role in control of endothelial function.

L.V. d'Uscio: None. Z.S. Katusic: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL131515.

Funding: No

Funding Component: P340

Molecular Mechanisms of Fetuin-a-induced Vascular Dysfunction and Inflammation: Role of Oxidative Stress and Toll-like Receptor 4

Augusto C Montezano, Delyth Graham, Rhian M Touyz, Inst of Cardiovascular and Medical Sciences, Glasgow, United Kingdom

Fetuin-A (FetA) is an endogenous agonist to toll-like receptor 4 (TLR4) and regulates insulin resistance and inflammation. FetA has been associated with endothelial dysfunction during metabolic diseases. Exact mechanisms whereby FetA influences vascular function in pathological conditions remain unknown, but we demonstrated that FetA regulates vascular function by Nox1 and TLR4 activation. Here we hypothesized that FetA, through changes in cell metabolism and activation of TLR4-Nox1 axis induces ROS formation and inflammation in hypertension. Normotensive (WKY) and hypertensive (SHRSP) vascular cells, as well as human microvascular endothelial cells, were stimulated with FetA (50 ng/mL). ROS production was measured by lucigenin and Amplex red, while gene expression was assessed by qPCR. FetA increased ROS production (131±49.2%), decreased H2O2 intracellular levels (63±14%) and increased gene
levels of IL6 (2 fold), IL1β (1 fold), RANTES (1 fold) and MMP2/9 (2 fold) in rat endothelial cells (vs. veh, p<0.05); all effects were blocked by TLR4 inhibitor (CLI095) and Nox1 inhibitor (ML171). FetA increased JNK (184±19%), but not p38 MAPK, activation in endothelial cells. In VSMCs, FetA-induced TLR4-dependent ROS generation was similar in WKY (136±9%) and SHRSP (144±14%) (p<0.05 vs veh). However, while IL6 gene expression was increased by FetA in WKY (4 fold) and SHRSP (0.5 fold), IL-1β gene levels were only increased by FetA in SHRSP (1 fold) derived VSMCs (p<0.05). CLI095 inhibited FetA effects on IL6 expression; however, TLR4 inhibition did not block FetA effects on IL-1β gene levels. In human endothelial cells, FetA increased ROS levels, was inhibited by CLI095 and a glucose-6-phosphate dehydrogenase (G6PD) inhibitor (6-aminonicotinamide), suggesting that FetA effects may be related to control of cell metabolism. In conclusion, FetA seems to regulate ROS and pro-inflammatory responses by TLR4, Nox1 and G6PD in endothelial cells. In VSMCs, FetA effects on oxidative stress and markers of cell injury are partially dependent on TLR4 activation and may involve other molecular partners.


Funding: No

Funding Component: P341

Osteoprotegerin (OPG) levels are increased in metabolic diseases, and are a biomarker of vascular dysfunction and cardiovascular risk. Mechanisms related to OPG-induced vascular dysfunction and its role in hypertension are not fully understood, but we previously demonstrated that OPG induces vascular dysfunction through ROS-dependent mechanisms. Here we assessed the molecular mechanisms whereby OPG regulates ROS and vascular function, with a focus on syndecan-1. VSMCs from normotensive (WKY) and hypertensive (SHRSP) rats were stimulated with OPG (50 ng/mL). ROS production was measured by lucigenin, amplex red and ELISA. In VSMCs from WKY rats, OPG increased ROS generation (158±15% vs veh, p<0.05). This effect was blocked by the syndecan-1 inhibitor (synstatin) and by removal of syndecan-1 sulfate proteoglycans side chains, chondroitinase and heparinase. OPG also increased H2O2 (2 fold) and ONOO− (1.5 fold) levels in VSMCs (p<0.05). H2O2 further stimulates ROS levels and redox signalling through activation of TRPM2, a redox-sensitive Ca2+ channel. TRPM2 inhibitors, 8-bromo-ADPR (8Br) and N-(p-amylcinnamoyl)anthranilic acid (ACA), did not block OPG-induced ROS generation in VSMCs from WKY rats. Syndecan-1 activation leads to FAK and c-Src activation, which are redox-sensitive signalling proteins. FAK, but not c-Src, activation (117±2%, p<0.05) was observed after OPG stimulation of WKY VSMCs. In VSMCs from SHRSP rats, OPG effects on ROS generation were exacerbated (230±40%, p<0.05) and inhibited by synstatin, 8Br and ACA. OPG also increased FAK (118±2) and c-Src (113±1) activation (p<0.05) in VSMCs from SHRSP rats. In conclusion, OPG regulation of oxidative stress is increased in hypertension and involves not only syndecan-1, but also TRPM2 channels, which may lead to activation of redox-sensitive proteins and vascular damage.

Funding: No

Funding Component: P342

Vascular Protein Oxidation and Redox Proteomics in Hypertension

Sofia Tsiropoulou, Augusto C Montezano, Inst of Cardiovascular and Medical Sciences, Glasgow, United Kingdom; Alan Scott, Richard J Burchmore, Inst of Infection, Immunity and Inflammation - Polyomics Facility, Glasgow, United Kingdom; Rhian M Touyz, Inst of Cardiovascular and Medical Sciences, Glasgow, United Kingdom

In hypertension (HTN) mechanisms whereby protein oxidation regulates vascular function remain unclear. We hypothesise that increased ROS promote a shift of oxidative post-translational protein modifications from reversible to irreversible forms, leading to aberrant redox signalling and vascular injury.

VSMC from mesenteric arteries of normotensive (WKY) and hypertensive (SHRSP) rats were stimulated with Ang II (10^{-7} M) in the presence/absence of PEG-catalase (1000U/ml) or tempol (10^{-5} M). Protein carbonylation was assessed by oxyblot and protein sulfenylation by DCP-Rho1 fluorescent probe. Protein tyrosine phosphatase (PTP)-oxidation, peroxiredoxin hyperoxidation (PRXSO3), γH2AX, Bcl2 levels were assessed by immunoblotting. DiGE and CyDye labelling screened for reversibly oxidised thiol proteome. Irreversible carbonylation and PRXSO3 were increased in SHRSP (fold change (FC)=1.29 and 2.77, p<0.05). Ang II-stimulation did not alter carbonylation levels. Reversible sulfenylation and thiol-proteome oxidation were reduced in SHRSP (FC=-1.18, p<0.05 and 13.6% (253 spots)). Ang II-treatment increased sulfenylation in WKY (FC=1.08, p<0.05) and SHRSP (FC=1.23, p<0.001); an effect inhibited by catalase. Reversible PTP oxidation was increased in WKY and SHRSP (FC=1.92 and 2.42, p<0.05), versus irreversible levels. Irreversible PTP oxidation tended to be higher in SHRSP. Ang II increased reversible PTP oxidation only in WKY (FC=1.27, p<0.05) and it was prevented by tempol. Ang II-stimulation increased protein levels of γH2AX (DNA damage) (FC=1.76, p<0.05) and Bcl2 (anti-apoptotic) (FC=2, p<0.05) in WKY. Proteomic data, filtered for FC>2, detected 1777 spots with 21% being differentially oxidised between WKY and SHRSP. Candidate proteins differentially oxidized between WKY and SHRSP include annexin A1 (-2.29) and galectin-1 (2.83). These results demonstrate altered redox status in HTN characterised by increased protein hyperoxidation and decreased reversible oxidation, in combination with decreased antioxidant capacity. Moreover, our findings identify novel candidate oxidized proteins implicated in VSMC motility, proliferation and signalling which may contribute to oxidative vascular injury in HTN.


Funding: No

Funding Component: P343

Exercise-induces Mitochondrial Remodeling Prevents Angiotensin II-induced High Blood Pressure

Joon-Young Park, Boa Kim, Satoru Eguchi, Victor Rizzo, Michael Brown, Temple Univ, Philadelphia, PA

Objective: Regular practice of exercise is one of the most effective non-pharmacological interventions that improve vascular health, which is thought to be mediated by a repeated exposure of vessel walls to increased
hemodynamic shear stress. The objective of this study was to investigate the effect of exercise preconditioning on endothelial mitochondria in an Ang II-induced hypertension model.

**Methods:** In *in vitro* experiments, human aortic endothelial cells (HAECs) pre-exposed to laminar shear stress (LSS) (20 dyne/cm², 48h) or situated under static flow were incubated with Ang II (100 nM, 2-6 h). In *in vivo* experiments, mice were singly housed with or without a voluntary running wheel for 7 weeks. Ang II (1 mg/kg per day) was infused using an osmotic pump for the last 2 weeks. Saline was used as a control.

**Results:** Significant increase in the expression levels of key genes related to mitochondrial biogenesis and dynamics were observed under shear stress conditions. Mitochondrial content determined by mtDNA content and immunostaining were higher (2-fold, \( P < 0.01 \)) in the shear-exposed cells. *En face* staining showed significantly higher mitochondrial content in the conduit and muscle feed arteries from exercise-trained mice compared with sedentary controls (\( N = 10, P < 0.01 \)) but not in the mesenteric arteries. Interestingly, LSS preconditioning attenuated Ang II-induced mitochondrial dysfunction in HAECs, which was evidenced by decreased mitoROS generation, increased \( \Delta \Psi_m \), and reduced mtDNA damage. Likewise, in aortic tissues, Ang II-induced mitochondrial phenotypic changes (i.e. mitoROS production, mtDNA damage and \( \Delta \Psi_m \) reduction) were significantly reduced in exercise-preconditioned mice compared to sedentary controls. Moreover, exercise preconditioning completely blocked Ang II-induced blood pressure elevation assessed by telemetry (MAP mmHg: 160.5 ± 1.5 vs 115.0 ± 11.4). **Conclusion:** Taken together, high-magnitude LSS improves endothelial function by enhancing mtDNA integrity and mitochondrial function. These findings further support the idea that aerobic exercise is a prominent life-style modification strategy to prevent hypertension by targeting dysfunctional mitochondria in the vessel wall.


**Funding:** Yes
**Funding Component:** National Center P344

**Increased Expression and Function of Endothelin Receptors in Aorta of (mRen2)27 Hypertensive Rats**

**Victor M Pulgar,** Oludamilola T Ademoyero,
Serguei S Sidach, Azeez A Aileru, Winston State State Univ, Winston Salem, NC

Endothelin-1 (ET-1) contributes to various cardiovascular diseases including hypertension. ET-1 is produced in the endothelium and acts via ETA receptors, located in smooth muscle cells, and via ETB receptors, located in both endothelium and smooth muscle cells. Activation of ETA produces vasoconstriction, whereas endothelial ETB activation mediates vasodilation, with previous studies showing that ET-1-mediated vasoconstriction is enhanced in hypertension. We hypothesized that increased ETA-mediated function is present in vasculature of the (mRen2)27 hypertensive rat. Western blotting of total protein extracts from thoracic aorta was used to determine expression of ETA and ETB from Sprague-Dawley (SD n=4) and (mRen2)27 hypertensive (n=5) rats. Specificity of western blot signals for ETA and ETB was assessed by using pre-absorption of primary antibody with the corresponding antigenic peptide and intensity of signals was measured by densitometry (NIH-J Image). Contractile responses to ET-1 (10⁻¹¹-10⁻⁷ M) in intact and denuded aorta were determined by wire myography (Multi Myograph, DMT-USA) in the presence of the ETA blocker BQ123 (10⁻⁶M) or the ETB blocker BQ788 (10⁻⁶M). Contraction to
ET-1 was expressed as maximal response (ET\textsubscript{MAX} as %K\textsubscript{MAX}) and sensitivity (pD\textsubscript{2}=-Log [EC\textsubscript{50}]). In aortae from (mRen2)\textsubscript{27} rats ETA and ET\textsubscript{B} receptor expression (48 and 31 kDa bands) was greater relative to aortae of SD rats (p<0.05). ET-1 contraction showed increased sensitivity in (mRen2)\textsubscript{27} vs SD aortae (pD\textsubscript{2}, 8.17±0.15 vs 7.76±0.12, p<0.05) with similar ET\textsubscript{MAX} (157±16 vs 145±6 %K\textsubscript{MAX}, p>0.05). In intact arteries, blockade of ET\textsubscript{B} increased ET-1 sensitivity (pD\textsubscript{2} 8.4±0.3, p<0.05) in SD without effect on intact arteries from (mRen2)\textsubscript{27} rats. In denuded arteries, ETB blockade increased ET\textsubscript{MAX} only in aortae from (mRen2)\textsubscript{27} (205±12 vs 152±5, p<0.05). Thus, increased ETA expression mediates greater ET-1-dependent contraction in vasculature of (mRen2)\textsubscript{27} rats. In (mRen2)\textsubscript{27} rats, loss of endothelial ET\textsubscript{B} receptor function in intact arteries may contribute to enhanced constrictor responses to ET-1, whereas increased ET\textsubscript{A} receptors in smooth muscle cells provide a counterbalancing vasodilation to offset maximal contractile effects of ET-1 in this model of hypertension.

V.M. Pulgar: None. O.T. Ademoyero: None. S.S. Sidach: None. A.A. Aileru: None.

Funding: No

Funding Component: P345

**Plasma Insulin Like Growth Factor Binding Protein-7 and Tissue Inhibitor of Metalloproteinase-2 are Associated with Reduced Renal Blood Flow and Change in Kidney Function After Revascularization in Human Atherosclerotic Renovascular Disease RVD**

Ahmed Saad, Sandra Herrmann, Hui Tang, John Woollard, Michael McKusick, Lilach Lerman, Stephen Textor, Mayo Clinic, Rochester, MN

(IGFBP-7) and tissue inhibitor of metalloproteinase-2 (TIMP-2) reflect G1-cell cycle arrest and are used as biomarkers for AKI. Recent studies show that these biomarkers rise in ischemic conditions and suggest that they actually may limit the severity of AKI. We tested the hypothesis that renal vein [IGFBP-7]*[TIMP-2] correlate with reductions in renal blood flow (RBF) and post-stent single kidney (SK)-GFR changes in patients with RVD undergoing contrast-based imaging and stent revascularization

Methods:

Inpatient studies were performed during 150 mEq Na+ intake and ACE/ARB Rx in patients with hemodynamically severe RVD (n=29, Doppler velocity = 318 ± 100cm/sec, and eGFR=34.7 ± 11.7 mL/ min) scheduled for renal artery stenting, and compared to essential hypertensive (EH) healthy controls (n=32). Cortical and medullary RBFs (by multidetector CT) and renal vein levels of IGFBP-7 and TIMP-2 were measured before renal artery stenting and 3 months later

Results:

Pre-stenting IGFBP-7 and TIMP-2 levels were elevated in RVD compared to EH (18.5±2. vs 15.7±1.5 and 97.4±23.1 vs 62.7±9.2 ng/mL respectively, P<0.0001). Baseline renal vein levels of IGFBP-7 * TIMP-2 correlated inversely with pre-stent RBF (r = -0.53, P=0.01) and directly with the change (%) in SK-GFR observed 3 months after stenting

Conclusion:

IGFBP-7 and TIMP-2 levels are elevated in patients with chronic RVD as a function of the baseline reduction in RBF, and associate with subsequent improvement in SK-GFR 3 months after revascularization. These data are consistent with renovascular occlusion inducing cell-cycle arrest that serves to protect kidney function.
Endothelial Function and NO Bioavailability are Improved in Angiotensin II-treated Transglutaminase-2 Knock-out Mice

Carmine Savoia, Emanuele Arrabito, Sergio Chiantotto, Carmine Nicoletti, Raffaella Carletti, Cira Di Gioia, Massimo Volpe, Sapienza Univ of Rome, Rome, Italy

We hypothesized that transglutaminase-2 (TG2) may contribute to the impaired functional properties of resistance arteries from angiotensin-II-treated mice. TG2-knockout mice (TG2-K/O, 12 weeks old, n=6) and wild type (WT) mice were treated or not with angiotensin-II (400ng/kg/min) for 14 days. Blood pressure (BP) and heart rate (HR) were measured by tail-cuff method. Endothelium-dependent and -independent relaxations were assessed by concentration-response curves to acetylcholine (1nM-to-100μM)±L-NAME (100μM) and sodium nitroprusside (10nM-to-1mM) respectively, in mesenteric arteries precontracted with norepinephrine (10μM). The expression of p-eNOS-(S1177)/eNOS, NOSIP (the negative modulator of eNOS), NOX-1, and its positive modulator ERp72 were evaluated in aorta by immunoblotting. Reactive oxygen species (ROS) production in aorta was evaluated by dihydroethidium staining. Plasma nitrate/nitrate were measured by ELISA. BP and HR were higher in TG2-K/O mice compared to WT (116.8±0.9 mmHg vs 89.6±1.5 mmHg, P<0.001; and 595.0±15.0 bpm vs 467.1±14.7 bpm, P<0.001, respectively). In both groups, angiotensin-II increased significantly BP (+28% in WT, and +21% in TG2-K/O) and HR (+33% in WT, and +9% in TG2-K/O). Acetylcholine-induced relaxation was preserved in WT and TG2-K/O and it was significantly impaired by angiotensin-II only in WT (-28%). L-NAME blunted this response in all the groups, although this effect was less evident in angiotensin-II-treated WT. Endothelium-independent relaxation was similar in all the groups. Plasma nitrates/nitrates and p-eNOS-(S1177)/eNOS were similar in WT and TG2-K/O, and they were reduced by angiotensin-II significantly only in WT (-37% and -44%, respectively). NOSIP expression was similar in both WT and TG2-K/O and was significantly increased by angiotensin-II only in WT (+40%). ROS production was similar in WT and TG2-K/O and significantly increased by angiotensin-II only in WT (+9%). NOX-1 and ERp72 were similar in WT and TG2-K/O and were significantly increased by angiotensin-II only in WT (+23% and +29%, respectively). In conclusion TG2 may contribute to endothelial dysfunction through the modulation of ROS production and the reduction of NO bioavailability in angiotensin-II infused mice.


Funding: No
Funding Component: P346

Retinol-binding Protein 7 (RBP7) is Required for PPARG-mediated Endothelial Protection via Adiponectin

PPARγ protects against endothelial dysfunction by regulation of unknown target genes. One such target, RBP7, an intracellular fatty acid-binding protein, exhibits endothelium-specific expression, but its effect on vascular function remain unknown. We hypothesize that RBP7 is endothelial protective. We examined vascular responses in basilar artery (pressurized myograph) of RBP7-knockout (KO) and wild type (WT) mice fed normal chow (ND) or high fat diet (HFD) for 8 wks. Endothelium-dependent acetylcholine (ACh)-induced relaxation was significantly impaired in HFD-fed KO mice (ACh, 100μM: 33±7% KO vs 83±10% WT, p<0.05), but not in ND-fed groups. This response was ameliorated by pre-incubation with superoxide scavenger tempol (1mM) or PEG-superoxide dismutase (100 U/ml). Mean arterial pressure (measured by radiotelemetry), body weight, hepatic steatosis, fasting glucose, glucose tolerance, and insulin sensitivity were similar in HFD-fed KO and WT mice. To identify targets downstream of RBP7, RNA-Sequencing was performed on carotid arteries from 8-week HFD-fed WT and KO mice as well as ND-fed age-matched littermates. Adiponectin (AdipoQ), a PPARγ target, was increased ~6-fold in HFD-fed WT mice, a response that was markedly blunted in KO mice. RNA sequencing was confirmed by qPCR. There was no difference in plasma AdipoQ. AdipoQ protein is expressed in endothelial cells of carotid arteries and its level of expression was increased in HFD-fed WT but not KO mice (AdipoQ/CD31: 1.14±0.1 WT-HFD vs 0.82±0.1 WT-ND, p<0.05; 0.79±0.1 KO-HFD vs 0.81±0.04 KO-ND). This led us to hypothesize that AdipoQ is involved in RBP7-mediated endothelial protection. Incubation of basilar artery with mouse full-length AdipoQ protein (5 μg/mL, 4 hours) significantly ameliorated endothelial dysfunction (ACh, 100 μM: 56±6% AdipoQ+KO vs 26±3% KO, p<0.05) and blunted carotid artery superoxide production in HFD-fed KO mice. AdipoQ also protects against endothelial dysfunction caused by subpressor Ang-II in KO mice. We conclude that RBP7 protects the endothelium from oxidative stress-induced dysfunction through an AdipoQ-dependent mechanism. Our evidence suggests RBP7 is an essential cofactor for activation of some PPARγ target genes in the endothelium.

C. Hu: None. H.L. Keen: None. K. Lu: None. D.R. Davis: None. X. Liu: None. J. Wu: None. S. Vogel: None. F.W. Quelle: A. Employment; Significant; University of Iowa. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH Grants. C.D. Sigmund: A. Employment; Significant; University of Iowa. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH Grants, AHA SFRN. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Significant; Carver Trust.

Funding: Yes
Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)
P600

Activation Of GPER Ameliorates Pulmonary Hypertension In Female Rats

Gisele Zapata-Sudo, Allan K Alencar, Daniele Gabriel-Costa, Ananssa M Silva, Guilherme C
Introduction: Pulmonary hypertension (PH) is primarily a disease of women (female-to-male ratio 4:1) and is associated with cardiac dysfunction. Aim: The activation of GPER by its agonist G1 was evaluated in monocrotaline (MCT)-induced PH rats. Methods: Depletion of estrogen was induced by bilateral oophorectomy (OVX; n = 18) in female Wistar rats (12 wks old). Experimental groups were: SHAM or OVX that received i.p. injection of MCT (60 mg/kg) for PH induction followed by administration of vehicle or G1 (400 µg/kg/day s.c.) for 14 days (n=7 per group). Hemodynamic parameters were determined by echocardiography. The effects of G1 in the maintenance of exercise capacity was investigated using a treadmill test. Results: MCT injection and estrogen loss led to a significant decrease in pulmonary acceleration time (PAT) and an increase in RV free wall thickness and MCT-related changes were attenuated by treatment with G1 ($P < 0.05$; Table 1). Right ventricular systolic pressure (RVSP) was higher in MCT-injected rats and the magnitude of this increase in OVX group was significantly higher than that in SHAM group. G1 normalized RVSP in both SHAM and OVX rats (Table 1). G1 treatment reversed altered expression of SERCA2a and phospholamban proteins in the RV (Table 1). Interaction between estrogen loss and MCT also reduced treadmill time to exhaustion in PH rats ($P < 0.05$), and chronic administration of G1 restored the exercise capacity ($P < 0.05$). Conclusion: G1 reversed PH-related cardiopulmonary dysfunction and exercise intolerance in female rats, a finding that may have important implications for the ongoing clinical evaluation of new drugs for the treatment of the disease in aging females.

### Table 1: Parameters of the experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>PAT (ms)</th>
<th>RVFWT (mm)</th>
<th>RVSP (mm Hg)</th>
<th>Treadmill time to exhaustion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>123.2 ± 13.2</td>
<td>1.21 ± 0.06</td>
<td>82.4 ± 2.24</td>
<td>122 ± 13.2</td>
</tr>
<tr>
<td>OVX</td>
<td>123.2 ± 13.2</td>
<td>1.21 ± 0.06</td>
<td>82.4 ± 2.24</td>
<td>122 ± 13.2</td>
</tr>
<tr>
<td>MCT</td>
<td>123.2 ± 13.2</td>
<td>1.21 ± 0.06</td>
<td>82.4 ± 2.24</td>
<td>122 ± 13.2</td>
</tr>
<tr>
<td>MCT+G1</td>
<td>123.2 ± 13.2</td>
<td>1.21 ± 0.06</td>
<td>82.4 ± 2.24</td>
<td>122 ± 13.2</td>
</tr>
</tbody>
</table>

G. Zapata-Sudo: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Instituto Nacional de Ciências e Tecnologia de Fármacos e Medicamentos (INCT-INOFAR).

A.K. Alencar: None. D. Gabriel-Costa: None. A.M. Silva: None. G.C. Montes: None. S.T. Martinez: None. A.M. Fraga: None. H. Wang: None. L. Groban: None. R.T. Sudo: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Instituto Nacional de Ciências e Tecnologia de Fármacos e Medicamentos (INCT-INOFAR).

Funding:

Funding Component: P601

Menopausal Female Mice are Hypersensitive to Pathological Cardiac Remodeling
Prior to menopause, women are protected against cardiovascular disease (CVD) compared to age-matched men; this protection is gradually lost after menopause. Loss of estrogen is detrimental; yet, estrogen replacement as cardioprotective remains controversial. Gonadectomized rodents demonstrate benefit of estrogen replacement for CVD. Human studies that show benefit of hormone replacement therapy are challenged by studies that do not show benefit. A novel model of menopause utilizing 4-vinylcyclohexene diepoxide (VCD) induces gradual ovarian failure, preserving the “perimenopause” transitional period and androgen secreting capacity of residual ovarian tissue. We hypothesize that menopausal females are hypersensitive to CVD-induced pathological cardiac remodeling. To address this hypothesis, we instigated menopause in 2 month-old females by daily (i.p.) injections of VCD (160mg/kg, 20 consecutive days); control mice received sesame oil as vehicle. Vaginal cytology was used to monitor estrous cycles and determine when cycling ceased. Mice were considered menopausal after 15 consecutive days in persistent diestrus, non-cycling. Female mice from cycling, perimenopausal and menopausal groups received the hypertensive agent angiotensin II (Ang II, 800 ng/kg/min via alzet s.q. mini-pump, 14 days). Menopause did not impact systolic blood pressure (SBP) measured via tail cuff (control: 115.1±6.0 mmHg, n=7; menopause: 115.0±5.2 mmHg, n=10). Ang II infusion induced a significant exacerbation of hypertension (SBP) in menopausal females (156.9±4.8 mmHg; n=10) compared to Ang II controls (114.7±5.0 mmHg; n=8). This hypersensitivity to Ang II-induced hypertension was attenuated by estrogen supplementation started during perimenopause (0.1 mg, 60 day release). In addition, menopausal females demonstrated worsened pathological cardiac remodeling measured by functional (echocardiography), cellular (myocardial fibrosis) and molecular (fetal gene program) assessments. Using a novel model of menopause (VCD) combined with infusion of the hypertensive agent Ang II, we demonstrated that menopausal mice are more susceptible, or hypersensitive to pathological cardiac remodeling compared to cycling and perimenopausal mice.


Funding:
Funding Component: P602

Reduced Symptoms And Improved Heart Rate Variability Associated With Use Of Closed-Loop Noninvasive Neurotechnology By Migraineurs

Catherine L Tegeler, Hossam A Shaltout, Charles H Tegeler, Wake Forest Univ, Winston Salem, NC

Introduction: Migraine is associated with impaired autonomic function, reduced heart rate variability (HRV), increased sympathetic activity, and symptoms of insomnia and depression. High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM) is a noninvasive, closed-loop acoustic stimulation neurotechnology that identifies dominant brain frequencies and translates them into audible tones, to support self-optimization of brain rhythms. Objective: We have reported use of HIRREM is associated with improved autonomic balance in a diverse cohort. We explored for effects of HIRREM in a cohort with self-reported migraine enrolled in
an IRB-approved open label feasibility study of HIRREM for diverse neuropsychological disorders. Methods: Fifty-two subjects (42 female), mean (SD) age 38.0 (18.6), received 15.9 (3.9) HIRREM sessions (90-120 minutes each) over 9.0 (2.7) days of in-office intervention. Outcomes collected before (V1), and 13.6 (14.4) days after HIRREM completion (V2) included measures of autonomic regulation (baroreflex sensitivity, BRS, and HRV), inventories for insomnia (ISI), depression (CES-D), traumatic stress (PCL-C), and headache (MIDAS). Paired t-tests were performed. Measures of BRS and HRV (n=52) improved from V1 to V2, including HF Alpha (+8.0 ms/mmHg (SE 2.2), p<0.0012), SDNN (+6.1 ms (1.9), p=0.002), and rMSSD ms (+7.6 (2.5), p=0.004). Sympathetic tone to blood vessels and mean arterial pressure were significantly reduced. There were improvements in symptoms at V2, including ISI (n=52): -6.2 (5.7), p<0.0001; CES-D (n=38): -8.0 (9.8), p<0.0001; PCL-C (n=30): -8.2 (11.3), p<0.001; and MIDAS (n=33): -14.9 (41.7), p<0.01. No serious adverse events were reported. Conclusions: This exploratory study shows improved measures of autonomic balance, reduced sympathetic tone, improved sleep and mood. Data suggest that HIRREM is a promising intervention that merits further investigation to mitigate the myriad effects of migraine.


Funding:

Funding Component: P603

**Lifetime Increase In Cerebral Angiotensin-(1-7) Induces Neuroprotection In A Model Of Brain Ischemia And Reperfusion**

Lucas M Kangussu, Ana F Almeida-Santos, Federal Univ of Minas Gerais, Belo Horizonte, Brazil; Michael Bader, Max-Delbruck Ctr for Molecular Med, Berlin, Germany; Andre R Massensini, Robson A Santos, Maria J Campagnole-Santos, Federal Univ of Minas Gerais, Belo Horizonte, Brazil

Recent studies showed that angiotensin-(1-7) [Ang-(1-7)] has cerebroprotective actions in ischemic and hemorrhagic stroke. Here we tested the hypothesis that transgenic rat (TGR-7371), which overexpress Ang-(1-7) in the brain, would exhibit neuroprotection in a model of brain ischemia/reperfusion by bilateral common carotid arteries occlusion (BCCAo). Evaluation of neurological deficit scores and bilateral asymmetry test (BAT) were performed 7 days after transient (25 min) BCCAo in TGR-7371 and Sprague-Dawley (SD) rats. The integrity of the blood-brain barrier (BBB) was assessed by the degree of extravasation of Evans blue dye (EB) intravenously injected and expressed as µg/100 mg of tissue. Cytokine levels were quantified in the whole brain through Elisa assay and expressed as pg/100 mg of tissue. Neurological deficits, such as ptosis palpebral, walking in circles, and/or ataxia, were observed in both SD-BCCAo and TGR-BCCAo, contrasting to sham-operated groups. However, TGR-BCCAo presented lower levels of cytokines (IL-1β: 125 ± 15; IL-6: 205 ± 27; TNF-α: 286 ± 6) when compared to SD-BCCAo. Levels of IL-10 were higher in SD-BCCAo than in SD control (112 ± 5 vs 29 ± 5) and even higher in TGR-BCCAo (172 ±
The present study shows that lifetime increase in cerebral expression of Ang-(1-7) induces neuroprotection in experimental global cerebral ischemia and reperfusion.


Funding:
Funding Component: P604

Astrocyte-dependent Regulation of Vascular Tone: Role in Hypertension

Juan Manuel Ramiro-Diaz, Ki Jung Kim, Jessica A Filosa, Augusta Univ, Augusta, GA

Clinical studies support that untreated hypertension (HT) accelerates the development of vascular cognitive impairment (VCI). Yet, the underlying mechanisms for VCI are not known. In a recent study we demonstrated the role of astrocytes in the regulation of parenchymal arteriole (PA) steady-state vascular tone. Here we hypothesized that hypertension results in structural and functional changes to the neurovascular unit resulting in enhanced astrocytic TRPV4 channel-dependent Ca²⁺ increases contributing to augmented pressure-induced PA constriction. Functional studies were conducted in brain slices from angiotensin II (AngII) treated mice (600 ng/Kg/min, 28 days). PA arterioles within brain slices were perfused and pressurized and myogenic-evoked diameter changes measured using video microscopy. In addition, using the GLAST-CreERT2; R26-Isl-GCaMP3 mice we measure myogenic-evoked Ca²⁺ changes in perivascular astrocytes. We demonstrate that HT increases pressure-induced PA tone by 11.14% at 30 mmHg and 12.97% at 60 mmHg (10.88 to 22.02 and 15.46 to 28.43% of tone, P<0.05 and P<0.01, respectively). In ANG II-treated mice, PA myogenic-evoked responses significantly increased astrocytic Ca²⁺ oscillations frequency (119.4%, 0.0366 to 0.0803 Hz, P<0.0001). A significant increase in astrocytic Ca²⁺ oscillation frequency was also observed after 2 min of AngII (500 nM) bath application (44.8%, 0.0366 to 0.053 Hz, P<0.01) in brain slices from AngII treated mice. Furthermore, using the model of spontaneous hypertensive rat (SHR) we observed that HT differentially increases vascular density and the number of vascular pericytes in cortical layers with highest neuronal densities (L III-V). Finally, while aquaporin 4 (AQP4) expression pattern was not different in the gray matter of SHR compared with WKY rats, a significant increase in unpolarized AQP4 expression was observed in the white matter of SHR. Taken together, this evidence indicates that HT induces functional and structural changes to the neurovascular unit favoring the development of regional brain hypoperfusion likely contributing to the development of VCI.

J. Ramiro-Diaz: None. K. Kim: None. J.A. Filosa: None.

Funding:
Funding Component: P605

Continuous Blood Pressure Parameter Calculated By The Optical Sensor Follows The Blood Pressure Value Changes Accompanied With The Treadmill Exercise

Madoka Yamazaki, Daito Bunka Univ, Higashimatsuyama, Japan; Hideo EDA, Grad. Sch for GPI, Hamamatsu, Japan

Introduction
Recently, it has been increased need for development and validation of non-mercury blood pressure (BP) measuring system with consideration for the environments. We developed a BP measuring system using an
optical sensor and reported that estimated BP value by the optical sensor (Opt BP) highly correlated with measured value of the BP with a mercury sphygmomanometer at rest (SBP r=0.93, DBP r=0.84) (Yamazaki and Eda, European society of Hypertension 2016). Purpose of this study is to validate whether the optical sensor can capture change in the blood pressure.

Methods
We conducted treadmill testing using Bruce protocol with 13 university students (male/female: 8/5). During the exercise and recovery stage, BP, heart rate and cardiac rhythm were recorded. The BP was measured with a cuff using auscultatory K sound every 1 or 2 minute automatically (Tango+, Sun Tech, U.S.A).

The optical sensor was attached the left index finger fixed with medical tape during the exercise. The data was sampled and stored in Windows PC within a second and the estimated BP value was displayed on the screen simultaneously. We compared the automatically measured BP (Auto BP) value and the Opt BP.

Results
Opt BP continuously showed the data also between the Auto BP measurements (Figure). Diastolic Opt BP was significantly higher (23mmHg) than the Auto BP in 10 of 13 subjects.

Conclusion
Continuous BP estimation by our optical system detected the BP change. The BP estimation at the higher sampling rate enables us to check the intermitted BP measurement value. Moreover, it is possible to store the estimated value more than 24 hours with a large memory device.

M. Yamazaki: None. H. Eda: G. Consultant/Advisory Board; Modest; ALPS Electric Co., LTD.

Funding:
Funding Component: P606 Cortisol Levels Correlate with Emotional Response to Perceived Racism in African American Males But Not Females

Mildred A Pointer, Sadiqa Yancey, Candace Wells, Jesus A Sanchez, Marilyn McClelland, North Carolina Central Univ, Durham, NC

Hypertensive African American (AA) males have as much as a 14-fold greater risk for kidney disease compared to others with hypertension. The reason for this greater risk is unknown. We have previously reported that stress contributes to resting blood pressure in normotensive AAs and can exacerbate the renal injury associated with hypertension in animal models. Consequently, we hypothesize that environmental stress activates the stress hormone system to a greater degree in AA males compared to females either due to either...
greater environmental stress (ES) exposure and/or greater stress hormone response to perceived ES. In this study we measured the level of perceived racism (PR), emotional response to PR (E), and baseline salivary cortisol (C) in normotensive AA males (n=39) and females (n=86). AA males had significantly higher PR scores compared to AA females for each of three domains: job (29 ± 3 vs 21 ± 2; \( p=0.005 \)); academic (27 ± 3 vs 20 ± 2; \( p<0.05 \)); and public (35 ± 3 vs 28 ± 2; \( p<0.05 \)). Although E and C did not differ between males and females (55 ± 3 vs 58 ± 2 and 0.2 ± 0.03 vs 0.2 ± 0.03, \( \mu g/dL \), respectively), E was significantly correlated with C in males (\( p=0.033 \)) only. There was no difference in age (25 ± 1 vs 23 ± 1, years) between the two sexes but systolic blood pressure was significantly higher in the males (121 ± 1 vs 114 ± 2, mmHg). We interpret these results to mean that ES is more likely to activate the stress system of AA males than that of AA females. Thus, in a culture where AA may experience greater unfair treatment in the job, academic, and public domains, activation of the stress hormone system in AA males may explain the disparity of kidney disease. The reason(s) for this sex difference in stress hormone response to perceived ES is intriguing and warrants further investigation.

M.A. Pointer: None. S. Yancey: None. C. Wells: None. J.A. Sanchez: None. M. McClelland: None.

Funding:
Funding Component: P607

Nitric Oxide (NO) Regulates Paracellular Permselectivity in Isolated, Perfused Rat Thick Ascending Limbs through Claudin-19

Casandra M Monzon, Jeffrey Garvin, CASE WESTERN RESERVE UNIVERSITY, Cleveland Heights, OH

About 50% of the Na reabsorbed in thick ascending limbs (TALs) traverses the paracellular pathway. The ionic selectivity of this route is regulated by claudins in the tight junctions. TALs express claudin-19 which has been reported to regulate TAL Na permeability. We showed that nitric oxide (NO) decreases Na/Cl permeability ratio (PNa/PCI) in TALs by increasing the absolute permeabilities of both ions though PCI increased more. However, whether NO affects paracellular permeability via claudin-19 is unknown. We hypothesize that NO regulates the paracellular permselectivity in TALs through this claudin. To test this we perfused TALs from Sprague Dawley rats and measured dilution potentials (a measure of permselectivity) with and without exogenously-added or endogenously-produced NO in the presence or absence of an antibody against an extracellular domain of claudin-19 or Tamm-Horsfall protein (control). Dilution potentials were generated by reducing bath NaCl from 141 to 32 mM. For the NO donor spermine NONOate (SPM): during the control period, the dilution potential was -9.3 ± 1.8 mV. After SPM (200 μM), it was -6.7 ± 1.6 mV (n = 6; \( p < 0.003 \)). In the presence of the claudin-19 antibody, SPM had no significant effect on dilution potentials (claudin-19 antibody alone: -12.7 ± 2.1 mV vs claudin-19 antibody + SPM: -12.9 ± 2.4 mV; n = 6). The claudin-19 antibody alone had no effect on dilution potentials. In the presence of the Tamm-Horsfall protein, the effect of SPM was still present (Tamm-Horsfall protein antibody alone: -12.7 ± 2.1 mV vs claudin-19 antibody + SPM: -12.9 ± 2.4 mV; n = 6). The claudin-19 antibody alone had no effect on dilution potentials. In the presence of the Tamm-Horsfall protein, the effect of SPM was still present (Tamm-Horsfall protein antibody alone: -9.7 ± 1.0 mV vs claudin-19 antibody + SPM: -6.3 ± 1.1 mV, \( p<0.006 \), n = 6). For experiments with endogenously-produced NO, L-arginine the substrate for NO synthase was added. During the control period, the dilution potential was -11.0 ± 1.1 mV. After L-arginine (500 μM) treatment, they were -9.0 ± 1.2 mV (n = 9; \( p < 0.05 \)). In the presence of the claudin-19 antibody, L-arginine had no significant effect on dilution potentials (claudin-19 antibody alone: -10.1 ± 0.9 mV vs claudin-19
antibody + L-arginine: -10.1 ± 1.0 mV; n = 9). In
the presence of the Tamm-Horsfall protein, the
effect of L-arginine was still present. We
conclude that the actions of NO on the
paracellular permselectivity in thick ascending
limbs are at least in part mediated by claudin-19.

C.M. Monzon: B. Research Grant (includes
principal investigator, collaborator, or
consultant and pending grants as well as grants
already received); Modest; HL28982. J. Garvin:
B. Research Grant (includes principal
investigator, collaborator, or consultant and
pending grants as well as grants already
received); Modest; HL28982.

Funding:
Funding Component: P608

Obesity Induced Hypertension Exacerbated
Through Upregulation Of Renal NaCI
Cotransporter, Reversed By Increasing Pgc-1α-
ho-1 Gene Expression To Restore
Mitochondrial Function

David Bamshad, Dept of Pharmacology, New
York Medical Coll, Valhalla, NY; Jian Cao, First
Dept of Geriatric Cardiology, Chinese PLA
General Hosp, Beijing, China; Joseph
Schragenheim, Charles T. Stier Jr., Dept of
Pharmacology, New York Medical Coll, Valhalla,
NY; Nader G. Abraham, Depts of Med and
Pharmacology, New York Medical Coll, Valhalla,
NY

Introduction: Hypertension caused by chronic
obesity as a result of high calorie food intake or
in leptin receptor deficient db/db mice may be
linked to mitochondrial dysfunction. Previously
we and others have shown that an
epoxycicosatrienoic acid agonist (EET-A),
reduced adiposity and ROS resulting in
normalization of BP by unknown mechanisms.
We hypothesize that EET-A will attenuate BP by
restoring mitochondrial function through
increasing the PGC-1α-HO-1 axis and increasing
urinary sodium excretion by downregulating
NCC channels.

Methods: Db/db mice at 16-wks of age were
divided into 3 treatment groups and for an
additional 16-wks received: A) control, B) EET-A
1.5mg/100g BW i.p. 2x/week and C) EET-A and
lentiviral (Ln)- PGC-1α shRNA (to suppress PGC-
1α protein). Oxygen consumption (VO₂), visceral
fat and blood glucose were determined.
Additionally, renal tissues were harvested to
measure the type 2 Na-K-Cl cotransporters
(NKCC2), epithelial Na channels- (ENaC), NaCl
cotransporters (NCC), PGC-1α, HO-1, insulin
receptors, and mitochondrial biogenesis
markers.

Results: At the conclusion of 32 weeks:
Group A, developed hypertension and
presented with decreased urinary Na excretion,
decreased VO₂, decreased downstream PGC-1α
signaling, and mitochondrial dysfunction. There
were increased levels of NCCs but not of
NKCC2s or ENaCs. Renal PGC-1α, HO-1, pAMPK,
and mitochondrial fusion protein Mfn 1/2, and
Opa1 were decreased, p<0.05.
Group B, exhibited restoration of renal levels of
PGC-1α, HO-1, pAMPK, and mitochondrial
biogenesis proteins Mfn 1/2 and Opa1. NCC
expression was reduced and was associated
with an increase in urinary Na excretion;
(p<0.05).
The beneficial effect of EET-A observed in group
B was suppressed in group C using Ln- PGC-1α
shRNA which suppressed PGC-1α expression in
renal tissue > 50% and was accompanied by the
onset of even more severe suppression of
urinary Na excretion than in Group A.

Conclusion: Treatment of obese mice with EET-
agonists leads to the recruitment of PGC-1α-
HO-1 which enhances mitochondrial function
and induces the downregulation of NCC
channels and increased sodium excretion. EET
may serve as a powerful therapeutic agent for
the treatment of obesity induced hypertension.
Saccharina Japonica Soaked In Vinegar Remarkably Decreased Blood Pressure In 2-kidney, 1-clip Renovascular Hypertensive Rats.

Saki Maruyama, Yukiko Segawa, Kobe Women’s Univ, Suma, Kobe, Japan; Hiroko Hashimoto, Osaka Seikei Junior Coll, Higashiyodogawa, Osaka, Japan; Tomoko Osera, Nobutaka Kurihara, Kobe Women’s Univ, Suma, Kobe, Japan

Objective: One of foods indispensable to Japanese cuisine “Washoku” is algae, including Saccharina japonica (SJ) and Undaria pinnatifida. The intake of SJ is reported to decrease blood pressure (BP) in spontaneously hypertensive rats in some studies, and in 2-kidney, 1-clip hypertensive (2K1C) rats in our studies. Since SJ soaked in vinegar is often used in Japanese cuisine, we observed the effects of dietary intake of SJ soaked in vinegar on BP in 2K1C rats.

Methods: Male Sprague-Dawley rats (6 wks) were treated with sham operation (SHAM) or clipping the left renal artery (2K1C). After surgery, the rats started receiving a control diet (C), a diet with 5.0% (w/w) SJ (S), or a diet with 5.0% SJ soaked in 5.0% (v/v) vinegar (SV). Systolic BP (SBP) was measured by a tail-cuff method every week for 6 weeks. At the end of the protocol, mean arterial BP (MAP) was measured in each rat under anesthesia.

Results and Discussion: Analysis of variance shows that SBP was significantly higher in 2K1C-C than SHAM-C through the experimental period (P<0.001), and that SBP was lower in 2K1C-S (P<0.05) and -SV (P<0.001) than in 2K1C-C (Fig). It also demonstrated that 2K1C-SV provided a significant reduction in SBP compared with 2K1C-S (p<0.001). At the end of the protocol, MAP in 2K1C-C was significantly higher than SHAM-C (154±4 vs 141±4 mmHg, P<0.05). Compared with 2K1C-C, a significant reduction in MAP was observed not in 2K1C-S (143±2 mmHg) but in 2K1C-SV (133±4 mmHg, P<0.05). Soaking in vinegar might bring alginate, which is one of possible components playing an important role in decreasing BP by SJ, to a low molecule and enhance the effect.

Conclusion: SJ soaked in vinegar may decrease BP more than SJ in 2K1C rats.

Hypertension Is Associated With Profound Pathological Changes In The Gut

YanFei Qi, Avinash Singh Mandloi, Monica M Santisteban, Vinayak Shenoy, Colleen T Cole-Jeffrey, Gilberto O Lobaton, Daniel Stewart,
Andres Rubiano, Chelsey Simmons, Michael J Katovich, Carl J Pepine, Mohan K Raizada, Univ of Florida, Gainesville, FL

Background and objective: Our previous studies have shown that gut microbial dysbiosis is linked to hypertension (HTN) in both animal models and patients with high blood pressure (BP) (Hypertension 2015; 65:1331-40). The intestinal epithelial layer serves as a barrier against pathogens and is altered in paracellular permeability with bowel diseases. Accordingly, our objective in the present study was to test the hypothesis that HTN-linked gut dysbiosis is associated with changes in gut wall pathophysiology. Methods and Results: Two rat models of were used: pre-hypertensive juvenile SHR (MAP 97 ± 5 mmHg) and adult SHR (MAP 160 ± 3 mmHg) with corresponding control WKY rats and chronic Angiotensin II (Ang II) infusion rat model of HTN (saline MAP 95 ± 2 mmHg vs. Ang II 150 ± 3 mmHg). A fluorescein isothiocyanate conjugated (FITC) dextran feeding protocol and a custom indentation system were used for permeability and stiffness assessments, respectively. Segments of small intestine were used for histology. We observed no significant differences in gut permeability and stiffness in pre-hypertensive juvenile SHR vs WKY controls. However, a 2-fold difference in gut permeability was observed in adult SHR (SHR 3514±563.4 vs WKY 1777±427.8, RFU, p<0.05). In addition, a 10-fold difference in gut wall stiffness (effective modulus) in adult SHR was observed (SHR 53.3±32.2 vs WKY 5.3±1.6, kPA, p<0.05). Histology revealed that adult SHR had 20% stunted villi length (SHR 580±54 vs WKY 718±6, μm, p<0.05) and a 60% increase in fibrotic area (SHR 13.8±1.3 vs WKY 8.5±0.8, p<0.05). Similar differences in gut permeability, elastic modulus, villi length and fibrotic area were observed in the Ang II HTN rat model. In contrast, pre-hypertensive, juvenile SHR did not show these differences. Conclusions: These observations, for the first time, demonstrate that increased BP is associated with profound gut pathology. They suggest that gut wall integrity likely plays a critical role in regulation of BP hemostasis and host microbiota communications with blood vessels.

Y. Qi: None. A.S. Mandloi: None. M.M. Santisteban: None. V. Shenoy: None. C.T. Cole-Jeffrey: None. G.O. Lobaton: None. D. Stewart: None. A. Rubiano: None. C. Simmons: None. M.J. Katovich: None. C.J. Pepine: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5UM1HL087366-09, 2UM1 HL087366-06. M.K. Raizada: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; R01 HL056921, R01 HL033610.

Funding:

Funding Component: P611

Immortalization of Sheep Proximal Tubule Cells Retain the Ability to Internalize Angiotensinogen that Traffics to the Mitochondria and Nucleus

Nildris Cruz-Diaz, Yixin Su, James C. Rose, Bryan A. Wilson, Mark C. Chappell, Wake Forest Sch of Med, Winston-Salem, NC

Although there is compelling evidence for an intracellular renin-angiotensin system (RAS) that includes localization of AT1, AT2 and AT7/Mas receptors (R) on the nucleus and mitochondria of various cell types, the mechanism for the intracellular expression of angiotensins remains equivocal as the precursor protein angiotensinogen (Aogen) enters the secretory pathway upon synthesis. Proximal tubules (PTs) of the kidney present a unique cell system since the PTs internalize Aogen and
transgenic mice lacking either the PT protein transporter megalin or liver Aogen exhibit reduced renal content of both Aogen and Ang II. We reported that isolated sheep PTs readily internalize Aogen, and subcellular fractionation revealed that Aogen was evident in the nuclear and mitochondrial fractions. The present study sought to establish a permanent cell line derived from the sheep PT to facilitate the characterization of Aogen internalization and processing. Sheep PT cells were isolated by protease digestion and Percoll density gradient separation, maintained in culture to promote epithelial cell growth and immortalized by SV-40 transfection. A clone (SPT-1) was obtained that expressed the SGLT-2 protein, a selective PT marker. SPT-1 cells were incubated with recombinant 125I-Aogen at 37°C in DMEM/F12 media. A time course [0.5 to 6 hrs] revealed linear uptake of Aogen \( r = 0.995 \) that did not saturate by 6 hrs. Pre-treatment of the SPT-1 cells with renin/ACE/peprilisin/chymase inhibitors [INHIB] or AT1R/AT2R/AT7/MasR antagonists [ANTAG] failed to attenuate Aogen internalization [Control: 209 ± 22; INHIB: 200 ± 21; ANTAG: 217 ± 15 fmol/hr/mg, n=3] while Ang II or Ang-(1-7) [10 µM, each] also did not inhibit, but tended to increase Aogen uptake [238 ± 24 and 244 ± 15 fmol/hr/mg, respectively, n=3]. Subcellular fractionation studies revealed that 12.0 ± 0.2% \( [n=3] \) of the total internalized Aogen was localized to the mitochondrial fraction with a higher content in the nucleus following an 18 hr uptake. We conclude that the established SPT-1 cell line which retains the capacity to internalize Aogen and expresses a similar pattern of protein trafficking to isolated PTs, may constitute a relevant model to elucidate the pathway for intracellular expression of angiotensins.


Funding:

The AA2-Ratio: Towards Improved Screening for Primary Aldosteronism

Marko Poglitsch, Attoquant Diagnostics GmbH, Vienna, Austria; Ashraf H Ahmed, Univ of Queensland, Brisbane, Australia; Andrea Stoller, Univ Hosp Basel, Basel, Switzerland; Dunja van Oyen, Oliver Domenig, Attoquant Diagnostics GmbH, Vienna, Austria; Manuel Haschke, Univ Hosp Basel, Basel, Switzerland; Michael Stowasser, Univ of Queensland, Brisbane, Australia

Background

Primary aldosteronism (PA) is a widely under-diagnosed, potentially curable and specifically treatable cause of hypertension. PA screening involves measuring the aldosterone-to-renin-ratio (ARR), but false negative results can occur in the setting of medications, which block the renin-angiotensin system (RAS). Withdrawing RAS blockers from patients with resistant hypertension is not without cardiovascular risk. A novel diagnostic approach, the aldosterone-to-angiotensin-II-ratio (AA2-Ratio), has the potential for less drug interference and improved reliability in PA screening and confirmation of diagnosis.

Methods

Serum samples from 80 patients undergoing PA confirmation testing were analyzed. Sampling was performed in a recumbent (7 a.m.) and in an upright (10 a.m.) position before and after 4 days of oral administration of fludrocortisone and salt loading. The concentrations of renin, aldosterone and equilibrium Angiotensin-II were determined and ARR and AA2-Ratios were calculated. The interference of ACE-inhibition with the AA2-Ratio was investigated in healthy volunteers receiving 10mg enalapril daily for 8 days.

Results

Renin concentration was undetectable in more
than 40% of samples, while equilibrium Angiotensin-II was measurable in 98% of all 320 samples analyzed. Angiotensin-II levels were significantly higher in upright collected samples compared to samples collected in a recumbent position. Comparison of the ARR with the AA2-Ratio revealed a significantly larger diagnostic window for the AA2-Ratio. While the ARR was significantly suppressed by ACE-inhibitor treatment, the AA2-Ratio remained unaffected by ACE-inhibition.

**Conclusion**
The AA2-Ratio may be superior to the ARR in PA screening among hypertensive patients. Equilibrium Angiotensin-II levels show expected responses to posture and appear to outperform renin concentration as a marker for RAS activation in terms of sensitivity, giving a measurable readout even in clinical states characterized by markedly suppressed RAS activity. The stability of the AA2-Ratio in the presence of ACE-inhibition points to a potential use of the AA2-Ratio PA screening in hypertensive patients without ACE-inhibitor discontinuation.


Funding:
Funding Component: P613

**Absolute Gene Quantification Of Intrarenal Renin-angiotensin System Components Using Droplet Digital PCR Analysis**

Ryousuke Satou, Akemi Katsurada, Kayoko Miyata, Andrei Derbenev, Andrea Zsombok, Dept of Physiology and Hypertension and Renal Ctr of Excellence, Tulane Univ Sch of Med, New Orleans, LA

The intrarenal renin-angiotensin system (RAS) has been shown to play crucial roles in the development of hypertension and RAS associated kidney injury including diabetic nephropathy. Although some circulating RAS components are filtered into kidneys and contribute to the regulation of intrarenal RAS activity, evaluating expression levels of RAS components in the kidney is important to elucidate the mechanisms underlying intrarenal RAS activation. Digital PCR is a new technique that has been established to quantify absolute target gene levels, which allows for comparisons of different gene levels. Thus, this study was performed to establish profiles of absolute gene copy numbers for intrarenal RAS components in wild-type (WT) rats, WT and streptozotocin (STZ)-induced diabetic mice. Male Sprague-Dawley rats (N=5) and male C57BL/6J mice were used in this study. The mice were subjected to either control (N=5) or STZ (200 mg/kg, N=4) injection. Seven days after STZ injection, copy numbers of renal cortical angiotensinogen (AGT), angiotensin-converting enzyme (ACE), ACE2, angiotensin type 1 receptor a (AT1a), and AT2 mRNA were determined by a droplet digital PCR. Since (pro)renin proteins produced by juxtaglomerular cells are secreted to circulating system, analysis of renin mRNA was excluded from this evaluation. In the renal cortex of WT rats, the copy number of AGT was higher than other measured RAS components (AGT: 719.2±46.6, ACE: 116.0±14.9, ACE2: 183.6±21.5, AT1a: 196.0±25.2 copies in 1 ng total RNA). AT2 levels were lower than other components (0.068±0.01 copies). In WT mice, ACE exhibited the highest copy number in the components (AGT: 447.2±29.0, ACE: 1662.4±61.2, ACE2: 676.8±41.5, AT1a: 867.0±16.8, AT2: 0.049±0.01 copies). Although STZ-induced diabetes did not
change ACE2 and AT1a, ACE levels were reduced (765.5±98.1 copies) and AT2 levels were augmented (0.10±0.01 copies) as previously demonstrated. Accordingly, the absolute quantification by digital PCR established precise gene profiles of intrarenal RAS components, which will provide rationales for targeting the each component in future studies. Furthermore, the results indicate that the high sensitive assay accurately quantifies rare target genes including intrarenal AT2.


Funding:
Funding Component: P614

Relationship Of Sodium Intake And Stress With Bone Health In Women

Allison Jasti, Deborah L Stewart, Gregory A Harshfield, Augusta Univ, Augusta, GA

**Background:** The skeleton is vital to sodium homeostasis, accounting for 40% of the body’s sodium. Research indicates stress and low sodium intake are independently associated with RAAS activation. In certain populations, stress can induce salt sensitivity, increasing the risk of hypertension and target organ damage, but the association of low versus high sodium intake with bone health is controversial.

**Purpose:** This study sought out the relationship of low sodium and stress-induced RAAS activation with bone health. The tested hypothesis was those with lowest sodium intake would have lower total bone mineral density (TBMD) and content (TBMC) associated with RAAS activation. In certain populations, stress can induce salt sensitivity, increasing the risk of hypertension and target organ damage, but the association of low versus high sodium intake with bone health is controversial.

**Methods:** We compared effect of stress on Ang II, Aldo, TBMD and TMBC in healthy Caucasian and African-American adolescents. Subjects were grouped by quartiles based on sodium intake, assessed by urinary sodium excretion.

**Results:** Due to females, overall significant inverse associations are observed between TBMD, TBMC, Ang II and Aldo in the lowest sodium intake quartile. Post-stress, women in the lowest sodium intake quartile showed that increases in both Ang II and Aldo correspond with lower TMBC and TMBD. There was no significance between Ang II, Aldo, TMBC and TMBD in the three highest quartiles of women nor in any male quartile.

**Conclusion:** These data suggest Ang II and Aldo may reduce TMBC and TMBD in women. Stress-induced increases in Ang II and Aldo, with low sodium intake, may further reduce TMBC and TBMC in women. Ang II inhibition and/or moderated salt intake may be an efficacious prevention or treatment against the development of osteoporosis.

<table>
<thead>
<tr>
<th>Lowest Quartile Sodium Intake Only</th>
<th>Across Genders</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBMC</td>
<td>TBMD</td>
</tr>
<tr>
<td>Stress</td>
<td>Ang II</td>
<td>-256°</td>
</tr>
<tr>
<td></td>
<td>Aldo</td>
<td>-236°</td>
</tr>
<tr>
<td>1-hour Post-Stress</td>
<td>Ang II</td>
<td>-353°</td>
</tr>
<tr>
<td></td>
<td>Aldo</td>
<td>-135°</td>
</tr>
<tr>
<td>2-hours Post-Stress</td>
<td>Ang II</td>
<td>-237°</td>
</tr>
<tr>
<td></td>
<td>Aldo</td>
<td>-183°</td>
</tr>
</tbody>
</table>

*Comparison resulted in different p-values for each paired analysis (n=61, b=64, c=57, d=52, e=60, f=63, g=56, h=51, i=33, j=32, k=28, m=36, n=31, o=29, p=27, q=30, r=50)


Funding:
Funding Component: P615

Stress-Induced Changes in Total Bone Mineral Content and Total Bone Mineral Density due to Activation of the Renin-Angiotensin-Aldosterone System in Adolescent Females

Vishwajeeth Pasham, Deborah Stewart, Laura Carbone, Gregory A Harshfield, Augusta Univ, Augusta, GA
**Background:** Previous literature has shown a strong negative effect of angiotensin II (ANGII) on bone metabolism within mouse models. Additionally, psychological stress has been associated with activation of the renin-angiotensin-aldosterone system (RAAS). Stress has also been related to lower total bone mineral density (TBMD). However, there is controversy in the literature examining the relationship between the RAAS and bone metabolism within humans and stress has not been considered as a direct link between these systems. **Purpose:** We aimed to examine the relationship between stress-induced RAAS activation and TBMD and total bone mineral content (TBMC). **Methods:** Participants were placed on a sodium controlled diet for three days. Participants then underwent two hours rest, one hour mental stressor, and two hours recovery with hourly collections of blood/urine samples. Renin, ANGII, aldosterone, TBMD and TBMC were measured. **Results:** This study recruited 586 adolescents (mean age 16±1.116) with 51% women and 62% African-American and 38% Caucasian. Overall, relationships were observed between ANGII and aldosterone, and TBMC and TBMD controlling for age, race, and BMI. During stress, aldosterone was related to TBMD (r=-.150, p<0.05) and ANGII was related to TBMC (r=-.156, p<0.05) and TBMD (r=-.139, p<0.05). When comparing males and females, only females demonstrated a relationship between TBMC and ANGII in response to stress (stress: r=-.229, p<0.05; post-stress: r=-.277, p<0.01) and between aldosterone and TBMC (stress: r=-.199, p<0.05) and TBMD (stress: r=-.250, p<0.01). Renin was not significantly correlated with TBMD nor TBMC in any population. **Conclusion/Interpretations:** These data suggest that stress-induced RAAS activation may be associated with lower TBMD and TBMC in girls. Despite small correlations, consistency across multiple measures of RAAS activation being apparent in adolescents is significant. This observation may indicate that stress activation of RAAS contributes to bone remodeling in early life.


**Funding:**

Funding Component: P616

**Evidence That The Extracellular Domain Of Na+/K⁺Atpase Is The Receptor For Cyclic Gmp-Induced Natriuresis**

Brandon A Kemp, John J Gildea, Nancy L Howell, Susanna R Keller, Robert M Carey, Univ of Virginia, Charlottesville, VA

Previous studies from our laboratory have shown that extracellular renal interstitial (RI) cyclic guanosine 3’5’-monophosphate (cGMP) increases urine sodium (Na⁺) excretion (Uₙₐᵥ) at the renal proximal tubule (RPT) in rats via activation of Src family kinase. Extracellular cGMP engenders this response through an unknown receptor. We hypothesized that cGMP binds to the extracellular domain of Na⁺/K⁺-ATPase (NKA) on basolateral membranes of RPT cells inhibiting Na⁺ transport. In the present study, we evaluated the effect of RI infusion of rostafuroxin (RF), a digitoxigenin derivative that specifically displaces ouabain (OUA) binding from NKA, on Uₙₐᵥ in the presence of RI cGMP infusion. Volume expanded, uninephrectomized, 12-week-old female Sprague-Dawley rats received RI infusions of vehicle (D₅W) (N=8), RI cGMP (18, 36, and 72 μg/kg/min; each dose for 30 min; N=10), or RI cGMP + RF (0.012 μg/kg/min; N=5) for 90 min following a 30 min control period with RI infusion of vehicle D₅W. RI cGMP infusion induced a significant natriuresis from 0.39 ± 0.06 μmol/min to 1.03 ± 0.21 (P<0.05), 1.17 ± 0.19 (P<0.01), and 1.94 ± 0.16 (P<0.001) μmol/min at 18, 36, and 72 μg/kg/min cGMP, respectively. RI co-infusion of cGMP + RF
abolished the cGMP-induced natriuresis at all doses (F=16.05, P<0.001). There was no change in mean arterial pressure during any infusion. To further demonstrate that cGMP binds to NKA, we performed a series of competitive binding studies in isolated RPTs from normal rat kidneys (N=4 for each) with bodipy-OUA (2 μM) + cGMP (10 μM) and 8-[Biotin]-AET-cGMP (2 μM) + OUA (10 μM). In the presence of cGMP, bodipy-OUA fluorescence intensity was reduced from 1422.1 ± 63 to 1072.5 ± 64 relative fluorescent units (RFU, P<0.01). In the presence of OUA, 8-[Biotin]-AET-cGMP staining was reduced from 1916.3 ± 144 to 1492.2 ± 84 RFU (P<0.05). Serving as control, biotinylated cAMP (N=2) did not demonstrate any fluorescence above background. Together, these data suggest that cGMP may compete with RF for binding on NKA and that the extracellular domain of NKA may serve as the receptor for cGMP-induced natriuresis.

B.A. Kemp: None. J.J. Gildea: None. N.L. Howell: None. S.R. Keller: None. R.M. Carey: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH RO1 Grant.

Funding:
Funding Component: P617

**Actions on the Splanchnic Venous System Contribute to 5-HT-induced Hypotension**

Bridget M. Seitz, Teresa Krieger-Burke, Hannah Garver, Gregory D Fink, Stephanie W Watts, Michigan State Univ, East Lansing, MI

Infusion of serotonin (5-hydroxytryptamine, 5-HT) into conscious normotensive and hypertensive rats causes a sustained reduction in systemic blood pressure. Imaging studies reveal that the blood pressure fall is closely associated with dilation of the large splanchnic veins (mesenteric, portal and abdominal vena cava), suggesting that active venodilation contributes to the fall in blood pressure. In fact, isolated splanchnic veins dilate directly to 5-HT via activation of the 5-HT7 receptor, and a 5-HT7 receptor antagonist prevents the 5-HT induced fall in blood pressure. To determine if the splanchnic venodilation caused by 5-HT is active or passive, anesthetized male Sprague Dawley rats were instrumented with arterial and venous lines for pressure measurements and 5-HT administration, respectively, while splanchnic veins were imaged using the Vevo® 2100 Ultrasound system. Measures were made relative to baselines measures. Within 5 minutes of infusion, 5-HT (25 ug/kg/min) caused an initial fall in portal vein pressure (~8-10% reduction) accompanied by dilation of the portal vein (~40% increase). No changes were seen in the dimensions or pressure of the abdominal vena cava at this time. Mean arterial blood pressure was reduced (>40% reduction). All of these changes were prevented by pretreatment with the 5-HT7 receptor antagonist SB269970. SB269970 during 5-HT infusion also caused an immediate reversal of changes in blood pressure and venous dimensions. Thus, active dilation of the prehepatic splanchnic venous system may be an early cause of 5-HT-induced hypotension. A more chronic experiment was performed in rats that were instrumented with a new dual channel radiotelemeter for concomitant measure of systemic and portal pressure in the conscious state. Within one hour after initiation of 5-HT infusion, portal venous pressure was elevated 38±0.2% above baseline (n=3) versus vehicle infused animals (4±0.3% above baseline; n=3), suggesting an action of 5-HT on intrahepatic venous resistance. Within 24 hours, portal pressure elevation resolved but blood pressure remained reduced. Collectively these data highlight the portal venous circulation as an important site of action for 5-
HT in causing acute and chronic falls in systemic blood pressure.

**B.M. Seitz**: None. **T. Krieger-Burke**: A. Employment; Significant; MSU. **H. Garver**: None. **G.D. Fink**: A. Employment; Significant; MSU. **S.W. Watts**: A. Employment; Significant; MSU. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Ionis Pharmaceuticals. G. Consultant/Advisory Board; Modest; Keystone SAB, PhRMA Foundation.

**Funding:**

Funding Component: P618

**Inactivation of Mitochondrial Deacetylase Sirt3 Promotes Vascular Oxidative Stress, Increases Endothelial Dysfunction and Exacerbates Hypertension**

**Anna E Dikalova, Roman Uzhachenko, Hana A Itani, David G Harrison, Sergey Dikalov, Vanderbilt Univ Medical Ctr, Nashville, TN**

Endothelial dysfunction is associated with aging, diabetes, hyperlipidemia, obesity and these risk factors affect the expression and activity of the mitochondrial deacetylase Sirt3. Sirt3 activates major antioxidant SOD2 by deacetylation of specific lysine residues and Sirt3 depletion increases oxidative stress. We hypothesized that loss of vascular Sirt3 increases endothelial dysfunction, promotes hypertension and end organ damage. The role of vascular Sirt3 was studied in wild-type C57Bl/6J mice and tamoxifen-inducible smooth muscle specific Sirt3 knockout mice (Smc\textsuperscript{Sirt3 KO}) using angiotensin II model of hypertension (Ang II, 0.7 mg/kg/day). Western blot showed 30% reduction of vascular Sirt3 and 2-fold increase in SOD2 acetylation in Ang II-infused WT mice. We have tested if ex vivo treatment of aorta with Sirt3 activator resveratrol improves endothelial function. Indeed, ex vivo incubation with resveratrol (10 \mu M) significantly reduced SOD2 acetylation, diminished mitochondrial O$_2$\textsuperscript{=} and increased endothelial NO to normal level while Sirt3-inactive analog dihydorresveratrol had no effect. Specific role of vascular Sirt3 was studied in Smc\textsuperscript{Sirt3 KO} mice by crossing floxed Sirt3 mice with mice carrying gene for inducible cre in the vascular smooth muscle. Sirt3 deletion exacerbates hypertension (165 mm Hg vs 155 mm Hg in wild-type) and significantly increases mortality in Ang II-infused Smc\textsuperscript{Sirt3 KO} mice (60% vs 10% in wild-type) associated with severe edema and aortic aneurysm (100% vs 20% in wild-type). Decrease of NO is a hallmark of endothelial dysfunction in hypertension due to vascular oxidative stress. Indeed, Ang II infusion increased vascular O$_2$\textsuperscript{=} by 2-fold and reduced endothelial NO by 2-fold. Interestingly, Ang II infusion in Smc\textsuperscript{Sirt3 KO} mice caused severe vascular oxidative stress (3-fold increase in O$_2$\textsuperscript{=}) and exacerbated endothelial dysfunction (4-fold decrease in NO). These data indicate that reduced vascular Sirt3 activity occurs in hypertension and this promotes vascular oxidative stress, increases endothelial dysfunction, exacerbates hypertension, increases end-organ-damage and mortality. It is conceivable that Sirt3 agonists and SOD2 mimetics may have therapeutic potential in cardiovascular disease.


**Funding:**

Funding Component: P619

**A Comparison of the Adrenergic System within the Perivascular Adipose Tissue in Two-High**
Fat Fed Models of Obesity-induced Hypertension: Dahl S and Sprague-Dawley Rats

Nadia Ayala-Lopez, Hannah Garver, Kyan Thelen, Robert Burnett, Andres Contreras, Gregory D. Fink, Michigan State Univ, East Lansing, MI; Stephanie W Watts 48824, Michigan State Univ, Michigan, MI

Increased sympathetic activity is one cause of obesity-induced hypertension. Over-activity of an adrenergic system in mesenteric perivascular adipose tissue (MPVAT) could contribute to high BP given its close proximity to splanchnic arteries and veins. We tested the hypothesis that high fat (HF) fed models of obesity-induced hypertension have elevated norepinephrine (NE) in MPVAT, increasing exposure of arteries to NE. Male Dahl S and Sprague-Dawley (SD) rats were fed a HF (60% fat, 0.3% NaCl; kcal) or a normal fat diet (NF; 10% fat, 0.25% NaCl; kcal) from weaning age (n=5). At 20-29 weeks of age, rats were sacrificed and tissues collected (all results shown in the table). HF increased the body weight of Dahl but not SD. Total fat mass was increased in the HF vs NF rats of both models. Mean arterial BP measured by radiotelemetry was elevated in the Dahl S HF vs NF and slightly elevated in the SD HF vs NF rats. Plasma NE was not elevated in either model. Surprisingly, MPVAT had significantly less NE in the Dahl S HF vs NF but was not altered in the SD. Expression of genes involved in NE synthesis, uptake and metabolism was measured by PCR to determine whether the MPVAT’s adrenergic system was altered in HF rats. Tyrosine hydroxylase (Th) mRNA was not detected in the Dahl S (SD not measured). Expression for the NE metabolizing enzymes monoamine oxidase-A (MAO-A) and catechol-o-methyl transferase (Comt) was not different. However, Sloc22a3 mRNA (organic cation transporter 3) was reduced in the SD HF vs NF rat. These data reveal that the elevation in BP in Dahl S and SD rats fed a HF diet may be due to a mechanism that is independent of elevated NE in PVAT. Funding: NIHP01HL70687, F31 HL12803501

<table>
<thead>
<tr>
<th>Physiological Parameters</th>
<th>Dahl S</th>
<th>HF</th>
<th>Dahl S</th>
<th>HF</th>
<th>Dahl S</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>273.4±4.9 (5)</td>
<td>793.6±4.9 (5)</td>
<td>273.4±4.9 (5)</td>
<td>793.6±4.9 (5)</td>
<td>430.8±4.9 (5)</td>
<td>470.2±4.9 (5)</td>
</tr>
<tr>
<td>Total fat weight (g)</td>
<td>12.6±2.5 (4)</td>
<td>10.8±2.5 (5)</td>
<td>12.6±2.5 (4)</td>
<td>10.8±2.5 (5)</td>
<td>12.6±2.5 (5)</td>
<td>12.6±2.5 (5)</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>101±2.3* (5)</td>
<td>101±2.3* (5)</td>
<td>101±2.3* (5)</td>
<td>101±2.3* (5)</td>
<td>101±2.3* (5)</td>
<td>101±2.3* (5)</td>
</tr>
<tr>
<td>Plasma NE (ng/ml)</td>
<td>483±40.2 (5)</td>
<td>308±40.2 (5)</td>
<td>483±40.2 (5)</td>
<td>308±40.2 (5)</td>
<td>483±40.2 (5)</td>
<td>308±40.2 (5)</td>
</tr>
<tr>
<td>MPVAT NE (ng/g)</td>
<td>30±440.2 (5)</td>
<td>112±440.2 (5)</td>
<td>30±440.2 (5)</td>
<td>112±440.2 (5)</td>
<td>50±440.2 (5)</td>
<td>50±440.2 (5)</td>
</tr>
</tbody>
</table>


Funding:
Funding Component: P620

Aging Decreases Vascular GPER Expression and Function

Sarah Lindsey, Jen L. Duong, Margaret A. Zimmerman, Tulane Univ Sch of Med, New Orleans, LA

Menopause accelerates the development of hypertension, arterial stiffness, end organ damage, and diastolic dysfunction. Postmenopausal hormone therapy relieves menopausal symptoms but promotes adverse cardiovascular outcomes, which may be due to aging-induced alterations in estrogen receptors. We previously published that GPER expression as well as agonist-induced vasorelaxation is decreased in mesenteric arteries from 12 month-old mRen2 female rats. We hypothesized that aging-induced GPER downregulation is present in other rodent species and strains as well. Aortas from young (2-3 months) and aged (11-23 months) C57BL/6 and BALB/c mice were lysed and immunoblotted for GPER protein. In addition, mesenteric arteries from C57BL/6 mice were
mounted on a wire myograph and assessed for vasorelaxation in response to estrogen receptor agonists. We found a significant downregulation of GPER in aging aortas (3.1 ± 0.6 vs. 0.63 ± 0.42, P=0.016, N=4 per group). Moreover, we found that vasodilation to the GPER agonist G-1 was significantly attenuated in aging resistance arteries (22 ± 7.5% vs 58 ± 6.5%, P=0.023, N=2-4). Interestingly, vasodilation to the nonselective receptor agonist estradiol was not altered by aging (24 ± 10% vs. 32 ± 2.0%, P=0.23, N=2-4). In light of our previous findings in the rat vasculature, our data indicate that aging-induced decreases in vascular GPER expression and function are conserved across vascular beds and rodent species. We propose that aging-induced GPER downregulation switches the vascular benefits of postmenopausal estrogen therapy from positive to negative. Our future goal is to determine whether therapies that target GPER improve cardiovascular outcomes to protect the aging female population.

S. Lindsey: None. J.L. Duong: None. M.A. Zimmerman: None.

Funding:

Funding Component: P621

Endothelial Mineralocorticoid Receptor Signaling Mediates Parenchymal Arteriole Hypertensive Remodeling and Cerebral Perfusion

Janice M Diaz-Otero, William F Jackson, Michigan State Univ, East Lansing, MI; Iris Z Jaffe, Tufts Medical Ctr, Boston, MA; Anne M Dorrance, Michigan State Univ, East Lansing, MI

Mineralocorticoid receptor (MR) activation causes hypertensive cerebral artery remodeling, but the vascular cell type involved has not been defined. Peripheral endothelial MR (EC-MR) activation mediates hypertensive artery dysfunction; the unique anatomy of cerebral arteries prevents the extrapolation of findings from the periphery to the brain. Parenchymal arterioles (PAs) perfuse the cerebral microcirculation and regulate cerebral perfusion. We hypothesized that EC-MR activation mediates inward PAs remodeling and reduces cerebral perfusion in hypertensive mice. To induce hypertension, male endothelial cell specific MR knockout (ECMRKO) and MR-intact littermates were treated with angiotensin II (AngII) (800ng/kg/min) for 4 weeks (n=6). Control mice were normotensive (n=6). PA structure, in 20-week-old mice, was assessed by pressure myography. Data are presented as mean ± SEM. In the absence of hypertension, EC-MR deletion did not change blood pressure, plasma aldosterone, cerebral perfusion or PA structure. AngII increased mean arterial pressure and plasma aldosterone in MR-intact and ECMRKO mice. Hypertension reduced cerebral perfusion in MR-intact mice but not in ECMRKO mice. In MR-intact mice hypertension caused inward hypertrophic remodeling evidenced by a decrease in the lumen diameter and wall area. These effects of hypertension where not observed in ECMRKO mice. (*p<0.05, Indicates significantly different from strain control). EC-MR activation mediates hypertensive remodeling in cerebral PAs; this was associated with a reduction in cerebral perfusion that could worsen the outcome of ischemic stroke or cause vascular dementia development.

Funding:
Funding Component: P622

Characterization of 125-I-Angiotensin (1-7) Binding to Rat Kidney

Filipe F Conti, Univ Nove de Julho, São Paulo, Brazil; Andrea Linares, Leena E Couling, Mariana Morris, Nova Southeastern Univ, Davie, FL; Katia De Angelis, Univ Nove de Julho, Sao Paulo, Brazil; Robert C Speth, Nova Southeastern Univ, Davie, FL

Despite the plethora of data indicating beneficial effects of angiotensin (1-7) (Ang 1-7) on the cardiovascular system, its putative receptor, Mas, has not been characterized in tissue membrane preparations other than single concentration demonstrations of the localization of 125I-Ang 1-7 binding sites in rat kidney. This does not indicate the specificity of 125I-Ang 1-7 binding nor does it indicate the actual densities of the binding sites, i.e., Bmax (fmol/mg tissue), or dissociation constant (Kd) to indicate binding affinity of 125I-Ang 1-7 for its putative receptor. To characterize 125I-Ang 1-7 binding in the kidney we prepared a low specific activity, monoradioiodinated Ang 1-7 using a 1:19 mix of 125iodine : 127iodine which allows for assessment of the Bmax and Kd with concentrations of radioligand up to 100 nM. Frozen kidneys from adult male albino rats were dissected and homogenized in water and the membranes were precipitated by centrifugation at 48 kxG. Membranes were resuspended in Tris:MgCl2 (50:1) pH 7.2 and incubated with 12 concentrations of 125/127I-Ang 1-7 ranging from ~3-100 nM for 30 min at 22 C, after which bound 125/127I-Ang 1-7 was resolved from unbound 125/127I-Ang 1-7 by filtration and measured with a gamma counter. Specific binding (defined as 100 µM Ang 1-7 displaceable binding) of 125/127I-Ang 1-7 showed a moderate binding affinity (Kd = 14.7 ± 1.8 nM) and binding site density (Bmax = 24.5 ± 9.9 fmol/mg initial wet weight). The Bmax value tended to be lower than that in the liver (Bmax = 62.3 ± 20.1 fmol/mg initial wet weight) and the Kd value was significantly greater (lower affinity) than that in the liver (Kd = 5.7 ± 0.6 nM, p = 0.0085). Of note, competition for 125/127I-Ang 1-7 binding Ang 1-7 indicated that the IC50 for Ang 1-7 competition for 125/127I-Ang 1-7 binding was 42.5 µM. Moreover, the ability of a variety of angiotensin peptides to inhibit 125/127I-Ang 1-7 binding at 100 µM, Ang 1-7 was less potent than the other angiotensin peptides: Ang III > Ang II > Ang I ~ Ang IV > Ang 2-7 > Ang 1-7 ~ Ang 3-7. These studies suggest that the binding site for 125/127I-Ang 1-7 is not specific for the putative Ang 1-7 receptor mas, and may represent a low affinity binding to the AT1 or AT2 receptor.


Funding:
Funding Component: P623

Endothelial Dysfunction and Abnormal Perivascular Adipose Tissue Signaling in Subcutaneous Vessels from HIV-infected individuals

Dan Wang 1, Cheng Wang 1, Hypertension, Kidney and Vascular Res Ctr. Georgetown Univ, Washington, DC; Cuiwei Wang 2, Chenglong Liu 2, Div of Infectious Disease and WIHS Program, Georgetown Univ, Washington, DC; Jennifer Verbesey 3, Transplant Inst, Georgetown Univ, Washington, DC; James Tomlinson 4, James Leiper 4, MRC Clinical Sciences Ctr, Imperial Coll, London, United Kingdom; Seble Kassaye 2, Div of Infectious Disease and WIHS Program, Georgetown Univ., Washington, DC; Mary Young 2, Div of Infectious Disease and WIHS Program, Georgetown Univ, Washington, DC;
Objective: Perivascular adipose tissue (PVAT) normally promotes vascular endothelial function, whereas is impaired by reactive oxygen species (ROS) in CVD. But PVAT effects on microvessels in HIV are unexplored. We previously reported endothelial dysfunction in living subcutaneous microvascular arterioles (SMAs) dissected from a gluteal biopsy in HIV infected individuals. We test hypothesis that HIV increases microvascular ROS and asymmetric dimethylarginine (ADMA) leading to impair microvascular function and PVAT signaling. Methods: Isolated SMAs with or without PVAT were prepared from young African American HIV-infected (n=8) and matched HIV-uninfected women (n=6) enrolled in the DC Women’s Interagency HIV Study (DC-WIHS). HIV-infected participants were virally suppressed on HAART without identified CVD risk factors (except obesity). SMA’s acetylcholine (ACh)-induced endothelium dependent relaxation (EDR), nitric oxide (NO) activity (DAF-FM), ACh-induced endothelium dependent contractions (EDC) and ROS generation (temp-9AC), plasma L-arginine, ADMA and adipose adipokines and malondialdehyde (MDA) were measured. Results: HIV-infected participants had significantly (p<0.05) reduced plasma ratio of L-arginine : ADMA (99 ± 13 vs 182 ± 32 µmol/µmol), increased adipose MDA (15.1 ± 2.5 vs 10.9 ± 2.6 ng/mg protein) and leptin (40 ± 9 vs 28 ± 7 ng/mg protein); and reduced adiponectin in plasma (14 ± 2 vs 23 ± 2 ng/ml) and in adipose (2.1 ± 0.3 vs 4.6 ± 1.3 ng/mg protein). ACh-induced EDR (57 ± 4 vs 71± 4%) and NO (0.20 ± 0.03 vs 0.58 ± 0.07 Δfluoresce unit) were significantly attenuated (p<0.05) in vessels from HIV-infected participants, whereas EDC (22 ± 2 vs 7 ± 2%) and O$_2^-$ (0.17 ± 0.02 vs 0.09 ± 0.02 Δfluoresce unit) were significantly increased (p<0.05). PVAT significantly increased (p<0.05) EDR (87 ± 3 vs 72 ± 4%) and NO (0.84 ± 0.09 vs 0.58 ± 0.08 Δfluoresce unit) only in control vessels. Conclusion: HIV-infected individuals have reductions in NO synthase substrate: inhibitor ratio (L-arginine:ADMA) and consequent increased intrinsic vascular effects of ROS leading to disruption of the beneficial microvascular PVAT signaling pathway. Therapeutic targets for vascular dysfunction in HIV should include ROS and its extravascular actions on ADMA and PVAT.

D. Wang: A. Employment; Significant; Georgetown University. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; WIHS, DC-FAR and Georgetown University. C. Wang: A. Employment; Significant; The third Hospital of Sun Yat-sen University, Guangzhou, China. C. Wang: None. C. Liu: None. J. Verbesey: None. J. Tomlinson: None. J. Leiper: None. S. Kassaye: None. M. Young: None. C.S. Wilcox: None.

Funding:

Funding Component: P624

Prevalence, Predictors and Comparison of Spironolactone versus Clonidine as a Fourth Drug for Resistant Hypertension: the Resistant Hypertension Optimal Treatment (ReHOT) study

Eduardo M Krieger, Luciano F. Drager, Dante M. Giorgi, Alexandre C. Pereira, Heart Inst (InCor), São Paulo, Brazil; José A. Barreto-Filho, Univ Federal de Sergipe, Aracaju, Brazil; Armando R. Nogueira, Univ Federal do Rio de Janeiro, Rio de Janeiro, Brazil; José G. Mill, Univ Federal do Espirito Santo, Vitoria, Brazil; José E. Krieger, Heart Inst (InCor), São Paulo, Brazil
Background: The prevalence, predictors and the best anti-hypertensive regimen for resistant hypertension (RH) are not well established especially in Countries with multiethnic profile. Our main aim was to compare spironolactone versus clonidine as a fourth drug therapy for patients with RH.

Methods: This is a multicentric, randomized controlled trial comprising 26 sites in Brazil that recruited outpatients from a highly admixed population with hypertension stage 2 (≥160/100mmHg) at study entry. Medical therapy adherence was checked by pill counting. Patients with confirmed RH (no office and 24hs ambulatory blood pressure monitoring - ABPM - control despite treatment with 3 drugs including a diuretic for 12 weeks) were randomized to additional 12 weeks treatment with spironolactone (12.5-50mg once daily) or clonidine (0.1-0.3mg twice daily). The primary endpoint was blood pressure (BP) control from both office (<140/90 mmHg) and 24hs ABPM (<130/80mmHg). Secondary endpoints included absolute and relative BP reductions in each study arm.

Results: A total of 1597 patients were included in the analysis. We found that 14.9% (238 patients) fulfilled the RH criteria. Predictors of true RH include male gender (OR 1.43; CI 1.02-2.00), previous stroke (OR 2.81; CI 1.51-5.06), diabetes (OR 2.09; CI 1.48-2.94) and BP ≥180x110mmHg at study entry (OR 2.53; CI 1.88-3.43). Compared to patients randomized to spironolactone (n=119), those patients randomized to clonidine (n=119) presented similar rate of the primary endpoint (19.8 vs. 24%, respectively; p=0.59). Similarly, no differences were observed between groups in the blood pressure reduction analyzed either by office as well as by 24-h ABPM. No differences in the pill counting monitoring were observed in the groups.

Conclusions: Appropriate treatment for stage 2 hypertension under the national universal health care conditions provided blood pressure control in 85% from a highly admixed population. Spironolactone or clonidine displayed comparable BP control as a fourth drug in patients with RH.

Funding: Ministry of Health/H. Samaritano, National Research Council, Sao Paulo Research Foundation and Zerbini Foundation.


Funding:

Funding Component: P625

Improvement of Autonomic Function and C-Reactive Protein in Military Personnel with Traumatic Stress After Use Of a Closed Loop Neurotechnology

Hossam A Shaltout, Catherine L Tegeler, Charles H Tegeler, Wake Forest Univ, Winston Salem, NC

Objective: Evaluate changes in autonomic cardiovascular control and inflammatory markers associated with use of High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM) in subjects enrolled in a pilot study for symptoms of military-related traumatic stress (MTS). Introduction: Symptoms associated with MTS include insomnia, depression, anxiety, activated inflammatory response and impaired autonomic control. HIRREM is a noninvasive, closed-loop acoustic stimulation technology that identifies dominant brain frequencies and translates them in real time into audible tones of variable pitch and timing, to support self-updating and self-optimization of brain activity. Methods: Eighteen service members or Veterans (1 female), mean (SD) age 40.9 (7.0), with MTS symptoms for 6 years (3.4), received 19.5 (1.1) HIRREM sessions over 12 days. Continuous recordings of blood pressure and heart rate, for analysis of baroreflex sensitivity (BRS) and heart
rate variability (HRV), were done before and immediately after completion of the HIRREM intervention. Blood samples were also collected (n = 14) for measurement of catecholamines, cytokines, C-reactive protein and the renin angiotensin system (RAS) components. Paired t-tests were performed. After HIRREM, there was improved BRS measured as HF alpha (9.6 ms/mmHg, SE 3.1, p = 0.005), Sequence Down (7.6 ms/mmHg, 2.4, p = 0.005), Sequence Up (8.4 ms/mmHg, 3.0, p = 0.01), and Sequence All (7.6 ms/mmHg, 2.2, p = 0.002), as well as HRV; SDNN (12.0 ms, 3.5, p = 0.02), rMSSD (13.2 ms, 3.0, p < 0.001), LF power (1023.0 ms², 346, p = 0.007), HF power (398.0 ms², 142.0, p = 0.01), and total power (1420.8 ms², 450.8, p = 0.005). C-reactive protein (36%, p = 0.057) was also reduced. There were no significant changes in the catecholamine, cytokines or RAS. There were no adverse events or dropouts.

Conclusions: These interim results suggest improved autonomic cardiovascular regulation, across multiple measures of BRS and HRV, and reduction in CRP associated with the use of HIRREM for symptoms of MTS. Confirmation of these results in a larger cohort may provide important insights regarding both the mechanisms associated with the beneficial effects of HIRREM, and the functional disturbances underlying MTS.

H.A. Shaltout: None. C.L. Tegeler: None. C.H. Tegeler: None.

Funding:
Funding Component: P626

**Stress-Induced Natriuresis and Blood Pressure Regulation in Patients with Meniere’s Disease**

**Seung Su Lee**, Deborah L Stewart, Gregory A Harshfield, Augusta Univ, Augusta, GA

**Objective:** Meniere’s disease is an idiopathic disorder characterized by strong attacks of vertigo, nausea, aural fullness, and fluctuating sensorineural hearing loss. Previous studies by our lab have suggested a possible role of stress-induced natriuresis and blood pressure regulation in Meniere’s disease. This study hypothesized patients with Meniere’s disease will excrete less sodium during stress, as compared to healthy individuals, and will have elevated and delayed blood pressure response.

**Methods:** Twenty-seven patients with Meniere’s disease (51.9% male; 61.1±11.5 years; 24 Caucasians, 3 African-American) were recruited for testing. Seventy-eight percent of the patients were taking medications including anti-inflammatory, diuretics, and anti-hypertensive medications. The protocol included 10 minutes rest, 20 minutes stress (competitive video game), and 10 minutes recovery. Urine samples were collected before and after the rest and stress periods. The data was compared to our previous study which has 586 healthy adolescents (49% male; 16.4±1.12 years; 222 Caucasians, 364 African-Americans) undergoing similar stress protocol.

**Results:** During stress, Meniere’s patients showed lower mean change in sodium excretion, as compared to healthy individuals (Mean deltaUNaV: 0.033±0.064 mEq/L vs 3.256±6.259 mEq/L with p=0.035, controlling for medication and age). A trend of elevated and delayed blood pressure response to stress in Meniere’s disease patients was also observed. Mean systolic blood pressure did not significantly change (p=0.798) in Meniere’s patients from stress (132.96±2.88 mmHg) to recovery (132.21±3.03 mmHg) while it changed significantly (p<0.01) from stress (115.83±0.48 mmHg) to recovery (110.02±0.41) in healthy individuals.

**Conclusion:** This study, to our knowledge, is the first to consider the stress-induced natriuresis among Meniere’s disease patients. Decreased sodium excretion with elevated and delayed blood pressure response, despite taking anti-hypertensive medications, suggests that angiotensin, an important stress hormone, may play a role in
the impaired natriuresis and blood pressure regulation in patients with Meniere’s disease.


Funding:
Funding Component: P627

Improved Autonomic Cardiovascular Regulation and Reduced Symptoms Associated with Use of Closed-loop Noninvasive Neurotechnology by Healthcare Workers

Catherine L Tegeler, Hossam A Shaltout, Lindsay I Howard, Charles H Tegeler, Wake Forest Univ, Winston Salem, NC

Introduction: Chronic stress in healthcare workers is associated with insomnia and risk for adverse health outcomes. High-resolution, relational, resonance-based, electroencephalic mirroring (HIRREM) is a noninvasive, closed-loop acoustic stimulation neurotechnology that identifies dominant brain frequencies and translates them into audible tones, to support auto-calibration and self-optimization of brain rhythms. Objective: We explore use of HIRREM in a cohort of healthcare workers enrolled in an IRB-approved open label feasibility study of HIRREM for diverse neuropsychological disorders. Methods: Twenty five employees (16 female), mean (SD) age 45.8 (13.9), received 14.8 (4.7) HIRREM sessions (90-120 minutes each) over 9.0 (3.6) days of in-office intervention. Data was collected before (V1), and 15.4 (11.7) days after completion (V2). Outcomes included BP and HR recordings for autonomic cardiovascular regulation (baroreflex sensitivity, BRS, and heart rate variability, HRV), with inventories for insomnia (ISI), depression (CES-D), traumatic stress (PCL-C), quality of life (EQ-5D global rating), and drop stick reaction testing (RXT). Paired t-tests were performed. Results: BRS and HRV (n=17) improved from V1 to V2, including Sequence ALL (+2.4 ms/mmHg (SE 2.8), p=0.15), SDNN (+8.9 ms (4.1), p=0.04), and rMSSD ms (+9.2 (4.9), p=0.07). There were significant improvements in symptoms and function at V2; ISI (n=25): -8 (5.3), p<0.0001; CES-D (n=20): -7.3 (13.4), p=0.02; PCL-C (n=19): -12.1 (12.4); p<0.001; EQ-5D (n=16): +10.8 (12.5), p<0.01; RXT (n=18): -4.6 cm (5.5), p<0.01. There were no serious adverse events. Conclusions: This exploratory study shows improved measures of cardiovascular regulation, and reduced insomnia, depression, and stress associated with use of HIRREM in a cohort of employees at an academic medical center. Data suggest that HIRREM is a promising intervention that merits further investigation to mitigate effects of chronic stress and improve wellness.


Funding:
Funding Component: P628

The Influence Of Religiosity On The Embracement Vs. Technology Based Distance Learning In Therapy Adherence In Patient Hypertensive

Grazia M Guerra, Heart Inst, Clinics Hosp, Sch of Med, Univ of São Paulo and São Camilo Univ Ctr, SP, Brazil, São Paulo, Brazil; Chao L. Wen, Telemedicine Dept, Sch of Med, Univ of São Paulo, SP, Brazil, São Paulo, Brazil; Margarida Vieira, Portuguese Catholic Univ, Oporto, Portugal, Cidade do Porto, Portugal; Isabela Fistarol, São Camilo Univ Ctr, São Paulo, SP, Brazil; Miriam H. Tsunemi, Inst of Mathematics and Statistics, Paulist State Univ (UNESP, in Portuguese) Botucatu, SP, Brazil, São Paulo, Brazil; Dante M Giorgi, Heart Inst, Clinics Hosp, Sch of Med, Univ of São Paulo, SP, Brazil, São Paulo, Brazil; Raquel A Motta, São Camilo Univ Ctr, São Paulo, SP, Brazil;
Jefferson C De Oliveira, São Camilo Univ Ctr, São Paulo, SP, Brazil, São Paulo, Brazil; Valéria Hong, Heno F Lopes, Fernanda M Consolim-Colombo, Luiz A Bortolotto, Heart Inst, Clinics Hosp, Sch of Med, Univ of São Paulo, SP, Brazil, São Paulo, Brazil

Introduction: The approach known as ‘embracement’ adopts relational strategies or soft technologies which promote bonding and may impact therapy adherence. Objectives: To assess the influence of the religiosity in the embracement approach on therapy adherence, quality of life, in hypertensive outpatients. This approach may be associated or not with the use of educational technology in a virtual learning environment (VLE) for distance learning (DL). Methods: This was a prospective randomized clinical study conducted with the following 3 groups of hypertensive patients: Group A (n=16, 12 women, mean age of 55.3±13 years, mean BMI of 32.3±6 kg/m², receiving individual orientation required by an embracement strategy characterized by 7 nursing visits at 20-day intervals, for 4 months); Group VLE (n=10, 7 women, mean age of 51.5±7 years, mean BMI of 29.4±6 kg/m², using a technological education strategy for DL and making 7 nursing visits at 20-day intervals, for 4 months); Control group (n=10, 5 women, mean age of 57.6±9 years, mean BMI of 29.7±6 kg/m², making 1 nursing visit at baseline and 1 after 120 days.) At baseline and after 120 days, the following tools were applied: the Morisky test, WHOQOL, Religion Index (DUREL), and ambulatory blood pressure monitoring (ABPM). The VLE group had remote access to the ‘Hypertension E-Care’ site (6 specific educational modules). Results: At baseline, there were no differences in clinical blood pressure, ABPM, and socio-demographic variables among the 3 groups. At the final assessment, the VLE group (44.4±0.4) showed significant improvement (p<0.05) in the social domain of quality of life when compared to group A (40.8±4) and the controls (41.9±3) groups. In therapy adherence (Morisky test), the VLE group showed significant improvement at the end of the study, which was not the case with the other two groups. The significant correlations were observed between index of religiosity and the differences of BP office for SBP R = -0.667, (p = 0.035 - negative correlation) and for DBP R = -0.666 (p = 0.035 - negative correlation) in VLE Group. Conclusion: This study shows that religious belief can improve blood pressure control, specifically when associated with education technology.


Funding:
Funding Component: P629

The Influence Of Religiosity Index And The Knowledge About Hypertension: How To Identify Indicators Of Patient With Blood Pressure Control

Jefferson C Oliveira RN, São Camilo Univ Ctrs, São Paulo, SP, Brazil; Luiz A Bortolotto, Hypertension Unit, Heart Inst (InCor, HC, FMUSP), Sch of Med, Univ of São Paulo, São Paulo, SP, Brasil, São Paulo, Brazil; Margarida M Vieira, Portuguese Catholic Univ, Port City, Portugal; Chao L Wen, Telemedicine Dept, Sch of Med, Univ of São Paulo, São Paulo, SP, Brasil, São Paulo, Brazil; Miriam H Tsunemi, Biostatistics Dept, Bioscience Inst, UNESP, SP, Brasil, São Paulo, Brazil; Dante M Giorgi, Valéria Hong, Hypertension Unit, Heart Inst (InCor, HC, FMUSP), Sch of Med, Univ of São Paulo, São Paulo, SP, Brasil, São Paulo,
Brazil; Isabela R Fistarol, São Camilo Univ Ctrs, São Paulo, SP, Brazil; Renato Chiavegato, Hypertension Unit, Heart Inst (InCor, HC, FMUSP), Sch of Med, Univ of São Paulo, São Paulo, SP, Brasil, São Paulo, Brazil; Grazia M Guerra, Hypertension Unit, Heart Inst (InCor, HC, FMUSP), Sch of Med, Univ of São Paulo, São Paulo, SP and São Camilo Univ Ctrs, São Paulo, SP, Brazil

Introduction: Can religious beliefs associated with the prior knowledge about the disease and treatment promote therapy adherence in hypertensive patients?

Objective: To identify association between the religiosity index (DUREL), level of education and performance on the knowledge test and blood pressure control.

Method: Cross-sectional study of a quantitative approach, were eligible 63 hypertensive patients for which knowledge tests were used, Morisky Green, and the Religion Index (DUREL). The research was approved by the Ethics and Research Committee. The surveys were applied on the occasion of the nursing consultation and measurement of blood pressure (BP) of Office and by Ambulatory Blood Pressure Monitoring (ABPM)

Results: Regarding the socio demographic characteristic predominated in this study: female 55.6%, the average age of 53.48±10 years, high school complete 31.7%, Catholic religion 79.4%, ethnicity 52.4% white, marital status married 66.7%, average BMI 30.14±5 kg/m². In BP Office showed average systolic blood pressure (SBP) 153.58±27 mm/Hg) and the diastolic blood pressure (DBP) 91.38 ± 15 mm/Hg. The mean values of SBP obtained with ABPM was 148.93±19 mm/Hg and 91.78±15 mm/Hg to DBP at day time, and 135.78 ±18 mm/Hg to SBP and 79.33 ±15 mm/Hg to DBP sleep time. In relation to the test of Morisky-Green to correlate with the values of the BP Office showed statistical significance (p = 0.004), for patients with therapy adherence in relation to the SBP; identified 20 adherent patients (score=4) vs non-adherent patients 43 (score ≤ 3). In relation to the blood pressure values of ABPM showed statistical significance also for SBP in the sleep time (p=0.000), and DBP (p= 0.017) the Morisky test. In relation the analysis of the performance test of prior knowledge and the blood pressure values, there was no statistical significance. Concerning the association of the Religiosity index with the values of the BP Office and ABPM there was no statistical significance.

Conclusion: The Index of Religiosity (DUREL) and the performance of prior knowledge about the disease and treatment were not sensitive to identify patients with better blood pressure control, unlike the Morisky Gren test, which was sensitive in identify patients with therapy adherence.

Funding:

Funding Component: P630

Nlpr3 Inflammasome Activation By Mitochondrial Dna Contributes To Oxidative Stress And Inflammation In The Vasculature Of Type 1 Diabetic Mice

Camila A Pereira, Nathanne S Ferreira, Camila Z Zanotto, Daniela Carlos, Rita C Tostes, Univ of Sao Paulo, Ribeirao Preto, Brazil

NLRP3 Inflammasome is a platform that regulates inflammatory responses by caspase-1 activation and processing of pro-IL-1β and pro-IL-18 to mature cytokines. NLRP3 is activated by several mechanisms including mitochondrial DNA (mitDNA). Circulating mitDNA is increased in diabetes, a condition associated with NLRP3 activation. We tested the hypothesis that
mitDNA release is increased in type 1 diabetes (T1D) leading to NLRP3 activation and contributing to vascular inflammatory and oxidative processes. Wild type (WT) and NLRP3-deficient (NLRP3−/−) mice were treated with vehicle or streptozotocin (40 mg/kg), i.p. for 5 days. Vascular reactivity was determined in mesenteric resistance arteries (MA). Cultured vascular smooth muscle cells (VSMC) were stimulated with mitDNA of T1D (dmDNA) and control (cmDNA) mice. Caspase-1 and IL-1β activation was evaluated by western blot analysis and reactive oxygen species (ROS) by fluorescence to DHE. DNA was extracted, purified and amplified by real-time-PCR. Data are presented as mean ± standard error of mean. Veh vs. T1D. NLRP3−/− T1D mice exhibited attenuated hyperglycemia vs. WT T1D mice [mg/dL, 241.0±27.7 vs. 337.6±18.1, p<0.05]. MA from T1D mice exhibited decreased ACh-induced dilatation vs. Veh [Emax, 46.6±4.0 vs. 91.5±2.8, p<0.05], which was not observed in NLRP3−/− T1D mice. Diabetes increased vascular caspase-1 [arbitrary units (a.u.), 1.2±0.1 vs. 0.8±0.5, p<0.05], but this activation was attenuated in NLRP3−/− T1D. T1D mice exhibited increased NLRP3 activation and mitDNA release in pancreatic cells and increased circulating mitDNA. dmDNA, but not cmDNA, increased NLRP3 activation in VSMC (i.e. activated caspase-1 and increased IL-1β levels) [a.u., 4.2±0.1 vs. 1.9±0.1; 2.3±0.1 vs. 0.7±0.1, p<0.05]. NLRP3 activation was attenuated in NLRP3−/− VMSC, but not in WT VSMC incubated with a TLR-9 antagonist. Increased ROS generation was observed in response to dmDNA, which was prevented by a mitochondrial uncoupler. Our data show that T1D increases mitDNA release, which promotes vascular NLRP3 activation via mitochondrial superoxide production, contributing to T1D-associated vascular dysfunction. Financial Support: FAPESP, CNPq.
glycosylation as a result of the high glucose treatment (p<0.05). Bioinformatics analyses of the targets revealed differential glycosylation of important endothelial cell surface receptors, a diverse array of ion channels, and immune regulators. The CD59 glycoprotein, important for protection from innate immunity, was increased in N-glycosylation (2.02 fold, p<0.05) and O-glycosylation (unique). Interestingly, glycosylation of CD59 is known to inhibit this protection from innate immunity. The lipoprotein receptor LRP1, important for leptin signaling and energy homeostasis, was also significantly increased in total N-glycosylation (1.8-fold total; p<0.05) and has been linked to insulin resistance. Overall, this study identified multiple hyperglycemia-induced differential glycosylations in the endothelium that may be key regulators of vascular dysfunction during the onset and progression of T2DM.

B.R. Hoffmann: None. M.E. Widlansky: None. A.S. Greene: None.

Funding:
Funding Component: P632

**Nlrp3/inflammasome Activation Contributes To Aldosterone-induced Vascular Dysfunction In Type 2 Diabetes.**

Nathanne S Ferreira, Thiago Bruder-Nascimento, Camila A Pereira, Camila Z Zanotto, Douglas S Prado, José C Alves-Filho, Daniela Carlos, Rita C Tostes, Univ of Sao Paulo, Ribeirao Preto, Brazil

Diabetic patients and animal models of type 2 diabetes (DM2) display increased plasma aldosterone (aldo) levels. Aldo induces vascular inflammation and endothelial dysfunction. NOD-like receptors, which are pattern recognition receptors involved in a variety of host innate immune responses, promote vascular inflammation. We hypothesized that aldosterone via mineralocorticoid receptors (MR) activates the inflammasome platform in the vasculature of DM2 mice. Control (db/+ ) and diabetic (db/db) mice were treated with vehicle or spironolactone (spiro - MR antagonist; 50 mg/Kg/day). Mesenteric resistance arteries (MA) from db/db mice exhibited reduced acetylcholine (ACh) dilation, which was reversed by spiro [Emax (% of relaxation): db/+: 78.5±4.1; db/db: 40.5±6.4; db/+spiro: 77.0±3.8; db/db+spiro: 62.8±5.9 n=3-6 p<0.05]. Spiro treatment reduced caspase-1 and mature IL-1β content in MA from db/db mice. Spiro also reduced caspase-1 activity in macrophages from peritoneal lavage of db/db mice [% of activity: db/+: 33.9±2.5; db/db: 51.8±7.4; db/+spiro: 31.1±1.9; db/db+spiro: 34.8±3.8 n=4-7, p<0.05]. In vitro, aldosterone increased mature IL-1β in vascular smooth muscle cells (VSMC) (cont: 0.9±0.01 ; LPS+Nigericine: 6.1±2.1 ; Aldo 4h: 9.7±2.6; LPS+Aldo 4h: 12.8±1.9 n=3-5, p<0.05). To determine whether aldosterone directly activates NLRP3/inflammasome in the vasculature and whether NLRP3 activation contributes to aldosterone-induced vascular injury, aldosterone was infused (600 ug/Kg/day for 14 days) in wild type (WT) and NLRP3 knockout mice (NLRP3-/-) after bone marrow transplantation from WT donor. The groups were constituted: WT->WT, WT->WT+aldo and WT->NLRP3-/->aldo. NLRP3 -/ mice were protected against aldosterone-induced endothelial dysfunction [Emax: WT: 89.3±2.9; WT+aldo: 39.8±1.8; NLRP3-/->aldo: 87.7±4.2, p<0.05]. Aldosterone treatment led to endothelial dysfunction in WT->WT mice, but WT->NLRP3--/ mice were protected from aldosterone-induced endothelial dysfunction [Emax: WT->WT: 95.1±3.1; WT->WT+aldo: 57.1±4.7; WT->NLRP3--/->aldo: 85.3±3.1 p<0.05]. These results suggest that NLRP3/inflammasome in the vasculature plays a crucial role on aldosterone/MR-induced vascular damage and on DM2-associated vascular dysfunction. Financial Support: FAPESP, CAPES, CNPq.
Resistance Training Counteracts The Systemic Catecholaminergic Hyperactivation Associated With Experimental Diabetes, But Not Normalize Cardiac Sympathetic Outflow

Ralmony A Santos, UNIFESP - Federal Univ of Sao Paulo, Jacareí, Brazil; Kleiton A Silva, UNIFESP - Federal Univ of Sao Paulo, Sao Paulo, Brazil; Juliana D Perez, UNIFESP - Federal Univ of Sao Paulo, Jacareí, Brazil; Nestor Schor, Dulce E Casarini, UNIFESP - Federal Univ of Sao Paulo, Sao Paulo, Brazil; Tatiana S Cunha, UNIFESP - Federal Univ of Sao Paulo, Sao Jose dos Campos, Brazil

Previous studies from our laboratory have demonstrated that chronic diabetes in rats results in cardiomyopathy, associated with sympathetic nervous system (SNS) hyperactivity. On the other hand, it is well known that the beneficial cardiovascular effects of exercise training in diabetes are due in part to normalization of the sympathetic outflow and improvement in the responsiveness of the myocardium to autonomic stimulation. Recently, resistance training (RT) has been recognized as a useful therapeutic tool for the treatment of chronic diseases and similar to aerobic exercise, has been reported to improve metabolic profile and body composition. Therefore, the aim of this study was to evaluate the effect of moderate-intensity RT on circulating and cardiac catecholamines concentration, to understand whether this type of exercise is also associated with cardiovascular protection. Wistar rats (3 months old) were randomized into: control (C), diabetic (D), diabetic + RPT (DR) and diabetic + APT (DA). Animals were made diabetic with a single tail injection of streptozotocin (STZ, 50 mg/Kg). Resistance exercise training was performed on a vertical ladder (5 days/week, 8 weeks) at 40-60% maximal load, and moderate aerobic training was performed on a treadmill (5 days/week, 8 weeks). Diabetes significantly increased plasma concentration of adrenaline (D: 5.3 ± 1.0 vs. C: 4.1 ± 0.6 ng/mL) and noradrenaline (D: 14.5 ± 0.2 vs. C: 3.1± 0.8 ng/mL), and both exercise modalities induced a significant reduction of them: adrenaline (DR: 1.1 ± 0.3; DA: 0.7 ± 0.16 vs. D: 5.3 ± 1.0 ng/mL) and noradrenaline (DR: 1.0 ± 0.2; DA: 0.7 ± 0.1 vs. D: 14.5 ± 0.2 ng/mL). Cardiac concentration of noradrenaline was also increased in diabetic group (D: 62 ± 7 vs. CS: 34 ± 6 pg/g) and only aerobic exercise was capable to reduce its concentration in heart tissue (DA: 30 ± 6 vs. D: 62 ± 7; DR: 55 ± 7 pg/g). The results from the present study show for the first time additional beneficial effects of RT on modulating SNS activity in diabetes. Moreover, considering that RT does not modulate cardiac catecholaminergic secretion, it also highlights the importance of aerobic training in diabetes treatment. Financial Support: FAPESP, CAPES, CNPq

Aerobic Training Prevents The Development Of Metabolic Abnormalities Induced By Chronic Stress, But Not Abnormal Circulating Levels Of Noradrenaline And Serotonin

Andrea Sanches, FOP/UNICAMP - Univ of Campinas, Piracicaba, Brazil; Juliana D Perez, UNIFESP - Federal Univ of Sao Paulo, Sao Paulo, Brazil
The chronic mild and unpredictable stress (CMS) protocol induces insulin resistance, dyslipidemia, oxidative stress and endothelial dysfunction in rats. Regular physical exercise is an effective non-pharmacological tool for the treatment of disorders induced by stress. The aim was to evaluate the role of physical training on hormonal and metabolic changes triggered by CMS. Forty male Sprague-Dawley rats were randomized into: Control, Stress, Exercise, Exercise + Stress, submitted to CMS protocol or to 8-week treadmill training (50-70% of the maximal exercise test). In the 4th, 5th and 6th wk, the animals were submitted to CMS protocol over seven days, repeating the procedures for 3 consecutive weeks. Two weeks after last stressor stimulus, blood and left ventricle were collected. Physical performance of animals submitted to the CMS was lower when compared to control animals, and physical training has not been able to alleviate this loss (p<0.05). The exercise prevented the development of metabolic changes induced by CMS, reducing hyperinsulinemia (Stress: 1.7±0.1 vs. Exercise: 1.4±0.1; Exercise + Stress: 1.3±0.1; Control: 1.4±0.0 ng/mL), insulin resistance index (Stress: 9.2±0.3 vs. Exercise: 7.1±0.2; Exercise + Stress: 5.8±0.2; Control: 8.1±0.2) and serum free fatty acids (Stress: 311.9±10.0 vs. Exercise: 193.0±16.7; Exercise + Stress: 251.6±14.16; Control: 190.1±17.3 mg/dL, p<0.05). In addition, it was also capable of reducing the cardiac concentration of serotonin (Stress: 3.1±0.1 vs. Exercise: 0.6±0.1; Exercise + Stress: 0.5±0.0; Control: 1.3±0.1 pg/g, p<0.05) of stressed animals (CMS). Physical training did not reduce the circulating concentration of noradrenaline (Stress: 603.7±52.2; Exercise: 83.8±10.8; Exercise + Stress: 748.9±46.1 vs. Control: 165.1±27.1 pg/mL) and serotonin (Stress: 1296.0±47.0; Exercise: 1196.0±68.1; Exercise + Stress: 1736.0±60.12 vs. Control: 619.8±79.6), which remained high in the groups submitted to the CMS (p>0.05). The results show that exercise improves metabolic losses triggered by CMS, but not the but not abnormal circulating levels of noradrenaline and serotonin, and suggest that physical training must be prescribed with caution to stressed individuals.

Funding:
Funding Component: P635

**Effect of Patient Characteristics and DPP4 genotype on Dipeptidyl Peptidase IV Activity and Inhibition by Sitagliptin**


Dipeptidyl peptidase IV (DPP4) inhibitors are a class of oral antihyperglycemic agents commonly used to treat type 2 diabetes mellitus (T2DM). Evidence suggests that these medications also have cardiovascular effects. **We tested the hypothesis that subject characteristics and genetic variability in DPP4, the gene encoding DPP4, affect response to DPP4 inhibition with sitagliptin.**

We studied 56 subjects undergoing trials of sitagliptin therapy. DPP4 activity was measured during placebo and after sitagliptin and percent inhibition calculated. Subject characteristics
were: 41.1% men (n=23), 19.6% African American (n=11), 30.4% with both T2DM and hypertension (HTN) (n=17), mean age 39.16±15.70 years, and mean BMI 26.71±6.24 kg/m². Sitagliptin doses were 100mg daily for 4-7 days (n=31) or a single dose of 200mg (n=25). Baseline DPP4 activity decreased significantly with age (r=-0.36, p<0.01) and was lower in T2DM HTN (22.54±5.20 vs 26.08±5.88U in non-diabetics, p=0.02). There was no effect of gender, race, weight, or BMI on baseline or inhibited DPP4 activity in T2DM or non-T2DM. Baseline DPP4 activity was significantly lower in rs4664446 (p=0.03; lower activity GG). These characteristics were not significant in a multivariable model. During sitagliptin, DPP4 activity was significantly higher (13.06±5.56 vs 6.85±3.34U, p<0.01) and percent inhibition lower (43±13% vs 74 ±11.2%, p<0.01) in T2DM HTN. Inhibited DPP4 activity declined with age in non-diabetics but not in T2DM. The variant rs2909451 was significantly associated with DPP4 activity during sitagliptin (p<0.01; higher activity TT). In multivariable analysis, inhibited DPP4 activity was associated with T2DM (p=0.02), rs2909451 genotype (p<0.01), but not age (p=0.08) or sitagliptin dose regimen (p=0.24). Inhibition of DPP4 by sitagliptin is decreased in T2DM HTN. Future studies are needed to determine if DPP4 inhibitor dose should be increased in T2DM HTN.

J.R. Wilson: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; T32KD007061. M.M. Shuey*: None. N.J. Brown: None. J.K. Devin: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; K23 HL119602 from NHLBI.

Funding:
Funding Component: P636

Insulin Resistance in Obesity Results in Postprandial Salt and Water Loss

Debra L Irsik, Ashley R. Washington, Rabei Alaisami, Michael W. Brands, Augusta Univ, Augusta, GA

Obesity and insulin resistance contribute to the development of metabolic syndrome, a growing epidemic in our country. The obese Zucker rat is an experimental model of this disease. Previously, using Sprague Dawley rats, we have shown that the normal postprandial rise in insulin acts physiologically to prevent renal salt and water wasting after meals. This study tested whether the effects of postprandial insulin would be attenuated in insulin resistant rats and result in excess salt and water loss. Chronic artery and vein catheters were implanted in male lean and obese Zucker rats for infusion and blood sampling. Rats were housed in metabolic cages and their catheters were connected to dual-channel Instech swivels for access. Over a 24-hr period of ad libitum eating, blood glucose was not different between obese and lean rats (127±7 vs. 120±3 mg/dl) but obese rats were hyperinsulinemic (14.86 vs. 0.98 ng/ml). Obese rats had significantly greater urine volume than lean controls (22.5±1.2 vs. 14.7±0.9 ml) despite similar water intakes. Obese rats tended to excrete more Na+ than lean controls (3.46±0.15 vs. 2.97±0.35 mEq) with equal amounts of Na+ intake. To evaluate the response to a single meal while controlling for blood glucose, fasted rats were administered a glucose bolus (as 50% dextrose) that yielded peak levels of blood glucose that were not different in the two groups (589±11 vs. 596 ±3 mg/dl at t=5 min.). Plasma insulin increased from fasting in both groups to 26.35 and 9.34 ng/ml in obese and lean controls, respectively. Over the 4-hour period following the glucose administration, obese rats had significantly greater urine...
volume (8.6±1.3 vs. 2.2±0.6 ml) and Na+ excretion (0.53±0.11 vs. 0.25±0.09 mEq) than lean controls. This suggests that insulin resistance of obesity may impair the ability of postprandial insulin to participate in maintenance of Na+ and water homeostasis, but the potential role of insulin resistance specifically within the kidney requires further study.


Funding:

Funding Component: P637

Absence Of Estrogen Receptor Alpha In T Regulatory Cells Results In Decreased Cardiac Glucose Uptake And Improves Diastolic Function In Western Diet-fed Female Mice

Camila Manrique, Guido Lastra, Annayya R Aroor, Dongqing Chen, Guanghong Jia, Lixin Ma, Susan C McKarns, James R Sowers, Univ of Missouri, Columbia, MO

Diabetic women are at greater risk of developing cardiovascular disease than diabetic men. Our group has shown that insulin resistant female mice are more prone to develop diastolic dysfunction than male mice when fed a high fat/high fructose (western diet - WD). T regulatory cells (Tregs) suppress inflammation and insulin resistance, and estrogen receptor alpha (ERα) signaling has been postulated to modulate their function. Consequently, using a novel rodent model lacking ERα in Treg cells (TregERαKO) we tested the hypothesis that in conditions of WD-feeding, abrogation of ERα signaling in Tregs in female mice results in worsened whole-body and cardiac-specific insulin sensitivity, as well as diastolic function. Female TregERαKO mice and ERαFloxed (ERαFl2) littermate controls were fed a WD for 16 weeks. WD consisted of high fat (46%) and high carbohydrate as sucrose (17.5%) and high fructose corn syrup (17.5%). At the end of the intervention, rodents underwent hyperinsulinemic-euglycemic clamps (n=7-9 per group) and, in a separate cohort, cardiac MRI (n=4 per group). Although TregERα deletion did not significantly impact whole-body insulin sensitivity (glucose infusion rate at steady state: 20±2 vs. 17±2 mg/Kg/min, TregERαKO vs. ERαFl2 respectively, p=0.22), cardiac glucose uptake was significantly lower in the TregERαKO cohort (214.0±7.5 vs. 167.3±19.6 μmol/min/100g tissue, TregERαKO and ERαFl2 respectively p<0.05) indicating cardiac insulin resistance. MRI analysis revealed no differences in systolic function between the cohorts. The initial filling rate (a marker of diastolic function) was greater in the WD-fed TregERαKO cohort (0.29±0.08 vs. 0.53±0.09 μL/ms, TregERαKO vs. ERαFl2 respectively, p<0.05) pointing toward enhanced diastolic function.

Conclusions: In female mice fed a WD, lack of ERα signaling in Tregs results in cardiac-specific insulin resistance. However, the decreased cardiac glucose uptake did not translate into deterioration of diastolic function. Further studies are needed to evaluate the differential effects of TregERα deletion on glucose uptake and diastolic function.

Acknowledgments: We acknowledge Vanderbilt MMPC for performing the clamps. MMPC is supported in part by grant U24 DK059637.

C. Manrique: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; K08HL129074-01. G. Lastra: None. A.R. Aroor: None. D. Chen: None. G. Jia: None. L. Ma: None. S.C. McKarns: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; R01-ES022966. J.R. Sowers: B. Research Grant (includes principal investigator, collaborator, or consultant and
pending grants as well as grants already received); Significant; R01-HL073101, R01-HL107910, 1BX001981.

Funding:
Funding Component: P638

**Muscadine Grape Seed Extract Improves Glucose Handling in Female but not Male Hypertensive (mRen2)27 Rats**

A’ja V. Duncan, Ellen N. Tommasi, Patricia E. Gallagher, E. Ann Tallant, Mark C. Chappell, Debra I. Diz, Wake Forest Sch of Med, Winston-Salem, NC

Muscadine grapes (*Vitis rotundifolia*) are enriched in polyphenols and other flavan-3-ols that may potentially convey cardiovascular benefit through the antioxidant properties of these compounds. In the current study, we established the effects of a muscadine grape extract (MGE, Piedmont Research and Development Corp.) on blood pressure and metabolic function in 20 week-old female and male hemizygous (mRen2)27 transgenic rats, an Ang II-AT1R-dependent model of hypertension. Littermates were treated with MGE (0.2 mg/mL) in the drinking water for 6 weeks (n = 7; male and n=5; female); controls were given water only (n = 7; male and n = 6; female). Intraperitoneal glucose tolerance test (IPGTT) assessed glucose metabolism and serum levels of glucose and insulin were also determined. There were no significant differences between the control and MGE-treated groups for either sex in systolic blood pressure (males: 168 ± 5 vs. 179 ± 4 mmHg; females: 183 ± 5 vs. 162 ± 11 mmHg) or body weight (males: 513 ± 12 vs. 508 ± 22 g; females: 297 ± 4 vs. 294 ± 89 g). The glucose response (area under the curve - AUC) in the female MGE-treated group was markedly lower compared to the untreated controls; however, MGE elicited no effect on the glucose AUC in males (see figure). Although MGE did not influence serum insulin AUC in males or females, the MGE-treated females exhibited a trend for a lower glucose-insulin index. We conclude that MGE intake improves glucose utilization in adult female hypertensive rats independent of changes in blood pressure or body weight. The mechanism(s) underlying the differential response to MGE between the female and male (mRen2)27 transgenic remain to be established.


Funding:
Funding Component: P639

**Sildenafil Treatment Improves Placental Levels of Placental Growth Factor in the Dahl Salt-Sensitive Pregnant Rat Model of Superimposed Preeclampsia**

Ana C Palei, Jennifer M Sasser, Joey P Granger, Univ of Mississippi Medical Ctr, Jackson, MS

Although the etiology of preeclampsia (PE) remains unclear, evidence indicates that impaired trophoblast invasion followed by placental ischemia promotes the release of placental anti-angiogenic factors into the maternal circulation. These factors then elicit maternal endothelial dysfunction and hypertension by blocking the action of molecules such as the placental growth factor
Inhibition of phosphodiesterase (PDE)-5 with sildenafil or other has been proposed as a potential therapy for PE; however, the mechanisms whereby PDE-5 inhibitors reduce blood pressure (BP) and improve uteroplacental perfusion during pregnancy are not clear. While previous studies have shown that PDE-5 inhibition induces PlGF production from human umbilical vein endothelial cells; it is unknown whether PDE-5 inhibitors also increase PlGF from placenta. Thus, the aim of this study was to evaluate whether sildenafil enhance placental secretion/production of PlGF in vitro and in vivo. In our in vitro protocol, we incubated placental villous explants from Sprague Dawley (SD) pregnant rats (n=4, 2-3 placentas per rat) at gestational day (GD)19 with different doses of sildenafil for 48h at 37°C under normoxia (8% O₂). PI GF-2 was measured in media of cultured explants by ELISA. We observed that sildenafil had no effect on PI GF-2 secretion from rat placental villi (vehicle: 562.7±46.6, 10nM: 559.3±39.5, 100nM: 556.4±35.9, 10μM: 546.2±37.5, and 100μM: 558.7±48.2pg/mg; P>0.05). In our in vivo protocol, we treated Dahl Salt-Sensitive (DS) pregnant rats (n=6-8 per group), which we had previously characterized as a model of superimposed PE, with sildenafil (50mg/kg per day, via food) from GD10 to 20. PI GF-2 was measured in placental homogenates by ELISA. While untreated DS dams exhibited an increase in BP and uterine artery resistance index (UARI) from baseline to late pregnancy, sildenafil-treated DS dams exhibited a significant decrease in BP and UARI. In addition, we found that placental levels of PI GF-2 were elevated in sildenafil-treated DS dams compared with untreated counterparts (1019±107.3 and 646.8±125.1pg/mg; P=0.0407). In conclusion, our findings suggest that the BP and UARI reduction in response to sildenafil may involve the indirect production of PI GF.


Funding:
Funding Component: P640

A Single-Chain Derivative of the Realaxin Hormone (B7-33) Protects Cytotrophoblasts from Hyperglycemia-Induced Preeclampsia Phenotype and Induces the Survival Pathway

Syeda H Afroze, Texas A&M Health Science Ctr Coll of Med, Temple, TX; Ahmed F Pantho, Univ of Texas, Austin, TX; Ram R Kalagiri, Thomas J Kuehl, Scott & White Healthcare/TAMHSC, Temple, TX; Ross Bathgate, Mohammed A Hossain, Florey Inst of Neuroscience and Mental Health, Univ of Melbourne, Melbourne, Australia; Mohammad N Uddin, Scott & White Healthcare/TAMHSC, Temple, TX

Background: Relaxin is a peptide hormone that allows vasodilation and plays an important role in the process of parturition. The literature suggests potential therapeutic role of H2 relaxin in preeclampsia (PreE), however, there is a controversy on hypotensive action of the peptide. Due to the complex insulin-like structure of relaxin (A- and B- chains, 53 amino acids, 3 disulfide bonds), a novel H2 relaxin B-chain-only peptide variant B7-33 (27 amino acids without any disulfide bonds) has recently been developed. This single-chain peptide displayed equivalent efficacy to the natural H2 relaxin in preeclampsia (PreE), however, there is a controversy on hypotensive action of the peptide. Due to the complex insulin-like structure of relaxin (A- and B- chains, 53 amino acids, 3 disulfide bonds), a novel H2 relaxin B-chain-only peptide variant B7-33 (27 amino acids without any disulfide bonds) has recently been developed. This single-chain peptide displayed equivalent efficacy to the natural H2 relaxin in several functional assays both in vitro and in vivo. Importantly, B7-33 was shown to have H2 relaxin-like RXFP1 specific effects, particularly in endogenously expressing RXFP1 cells, thus we hypothesized that B7-33 could be an alternative and cost-effective treatment option for PreE compared with H2 relaxin.

Methods: Human CTBs were treated with 100, 150, 200, 300, or 400 mg/dL glucose for 48h and were co-treated with B7-33 (25 nM) with glucose exposure, while some cells were
Levels of vascular endothelial growth factor (VEGF), placental growth factor (PIGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and soluble endoglin (sEng) were measured in culture media using ELISA kits. Cell lysates were utilized to evaluate the mTOR, pAKT and total AKT expression by western blotting. Statistical comparisons were performed using analysis of variance with Duncan’s post hoc test. Results: Secretion of sFlt-1 and sEng were increased while VEGF and PIGF were decreased in CTBs treated with ≥150 mg/dl of glucose (*p < 0.05 for each). B7-33 co-treatment significantly rescued CTBs from hyperglycemia-induced anti-angiogenic profile (p < 0.05 for each). There is no effect of B7-33 on sFLT-1, sEng and PIGF; however, it increases expression of VEGF, while CTBs were treated only with B7-33. B7-33 also causes increased mTOR and pAKT expression in CTBs without any change in total AKT. Conclusions: B7-33 mitigates the hyperglycemia-induced dysfunction of CTBs by attenuating anti-angiogenic phenotype similar to that seen in PreE. This study supports the importance of continuing research of B7-33 in preE prevention.


Funding:

Funding Component: P641

Markers of Autophagy are Induced in Response to Placental Ischemia

Adrian C Eddy, London J Williams, Heather Chapman, Eric M George, Univ of MS Medical Ctr, Jackson, MS

Preeclampsia is a disorder characterized by new onset hypertension during pregnancy, as well as other clinical findings such as proteinuria and edema. This disorder affects approximately five percent of pregnancies and is the leading cause of maternal and fetal morbidity. Though the origins of the disease are unclear, it is thought that placental ischemia is central to its etiology. Commonly, the uterine spiral artery fails to adequately remodel to allow for adequate blood flow to the developing fetal/placental unit, causing hypoxia and ischemia. It is well established that hypoxia can lead to endoplasmic reticulum (ER) stress in cells. When ER stress occurs, protein misfolding occurs, leading to the unfolded protein response (UPR). One process which could be important in an attempt to restore cellular homeostasis is autophagy, a specialized process of carrying defective proteins to lysosomes for degradation to prevent aggregation from occurring. There are several factors which play a role in this process, and are utilized as a marker for autophagy, notably Beclin-1 and various autophagy related genes (Atg). Though autophagy has been examined previously in cancer and numerous cardiovascular abnormalities, it is unclear whether induction of autophagy is one of the cell survival pathways activated in response to placental ischemia. Here we have examined placental tissue from term pregnant rats and rats which have undergone placental ischemia due to the reduced uterine perfusion pressure (RUPP) procedure to to determine whether autophagy pathways have been activated as part of the cell survival response during chronic ischemia. As determined by western blot, protein levels of the autophagy markers beclin (57 ± 6 A.U. vs 113 ± 16 A.U., p<0.05), Atg3 (34 ±3 A.U. vs 54 ±8 A.U., p<0.05), Atg12 (550 ± 77 A.U. vs. 1022 ± 160 A.U., p<0.05), and Atg16 (43 ± 6 A.U. vs 147 ±47 A.U., p<0.05) were all significantly increased in placental tissue in RUPP treated animals. These data, coupled with our previous studies, suggest that several cell survival pathways are activated in response to chronic placental ischemia, notably programmed cell
autophagy. The importance of these pathways in survival of placental tissue and overall placental function will be the object of future study.


Funding:
Funding Component: P642

Two Pools of Eicosatrienoic Acids in Humans: Alterations in Salt Sensitive Normotensive Subjects


We measured eicosatrienoic acids in 21 normotensive subjects classified as salt-resistant (SR=13) or salt-sensitive (SS=8) with the rapid protocol of Indiana University (high salt, HS=460 mEq Na/24 hrs, low-salt, LS=10 mEq Na/24 hrs + furosemide 40 mg x 3). No EETs were detected in urine; hence, ELISA 14-15 DHETs were taken to represent the total pool of this urine isoform (U-TP). Plasma total pools (8-9,11-12,14-15 and 14-15 P-TP) are the sum of EETs+DHETs (HPLC-MS), and their results were similar, so the former are reported. Analyses required log-transformation of not normally distributed data. U-TP was not changed by HS but decreased with LS (Δlog -0.47±0.19, p<0.01), consistent with the response of a natriuretic system. Further, in the baseline, HS and LS periods combined, U-TP correlated positively with UNaV (r=0.35, p<0.005), fractional excretion of Na (r=0.37, p<0.003) and Na/K ratio (r=0.39, p<0.002), indicating inhibition of ENaC. P-TP was not changed by HS (due to inhibited soluble epoxide hydrolase with reduction in DHETs and increase in EETs) but was increased by LS (Δlog 0.05±0.02, p<0.01), including EETs (0.04±0.02, p<0.03), inconsistent with a natriuretic system. P-TP did not correlate with urine parameters. Instead, plasma DHETs correlated with aldosterone (r=0.34, p<0.005) and plasma EETs with catecholamines (r=0.45, p<0.001). Differences between SR and SS subjects included: a) lower levels of U-TP and P-TP in SS than SR, significant in some stages of the experiment, b) lack of response of U-TP to changes in salt balance in SS, c) lack of the correlations between U-TP and natriuresis/ENaC activity and between DHET/aldosterone in SS, all observed only in SR. We conclude that: 1. Urine eicosatrienoic acids reflect a renal pool involved in regulation of natriuresis whereas plasma ones are probably of systemic origin and uninvolved in Na excretion, 2. There may be a feed-forward mechanism for the systemic, non-renal effects of aldosterone, by stimulation of inactive DHETs, 3. Catecholamines may stimulate epoxygenases or EETs may produce neuronal release of catecholamines, which remains to be investigated, and 4. Differences between SS and SR suggest abnormalities of eicosatrienoic acid regulation of natriuresis in SS subjects.


Funding:
Funding Component: P643

Potassium Differential Stress Response with Angiotensin II Blockade in Blacks

Deborah L Stewart, Gregory A Harshfield, Augusta Univ, Augusta, GA; Coral Hanevold, Univ of Washington, Seattle, WA; Sunil Mathur, Augusta Univ, Augusta, GA

Background: In youths, we demonstrated stress increases systolic blood pressure (SBP) with.
decreased urinary sodium excretion (UNaV) and no change in urinary potassium excretion (UKV) in retainers (retain sodium during stress) but increased UNaV and UKV in excreters (excrete sodium during stress). Angiotensin receptor blockers (ARBs) are known to increase UNaV, yet are not commonly used in blacks. **Purpose:** This study sought to determine if angiotensin II plays a role in the Na/K imbalance in retainers. The tested hypothesis was angiotensin II (Ang II) blockade stabilizes the urinary sodium/potassium (UNa/KV) excretion ratio during stress. **Methods:** Impact of Ang II on changes in SBP, UNaV, UKV and UNa/KV ratio in response to stress was tested in blacks after three day sodium/potassium controlled diet in a double-blind, placebo-controlled crossover design using irbesartan. **Results:** Excreters (n=84) and retainers (n=25) were identified and compared on placebo versus ARB. In response to Ang II blockade, excreters reduced their UNa/KV ratio and increased UNaV. Conversely, retainers did not change UNaV while sustaining their UNa/KV ratio. However, both groups increased SBP and UKV with a greater effect in excreters. **Conclusion:** These data suggest Ang II plays a significant role in the UNa/KV ratio homeostasis, through its impact on UNaV. Specifically, retainers are able to maintain their UNa/KV ratio during stress with Ang II blockade, whereas excreters are unable to maintain their UNa/KV ratio under the same treatment. Identification of this stress response pattern may be a critical need in providing personalized and effective treatment.


**Funding:**

Funding Component: