Gastro-renal Communication: Role in Hypertension

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Faculty Disclosure

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Gastro-renal communication: role in hypertension

Financial Disclosure:
Pedro A. Jose is co-owner of Hypogen, Inc. which owns the patent for G protein-coupled receptor kinase 4 (GRK4)
It is a great honor to be a co-recipient of the 2015 Excellence Award for Hypertension Research.

Thank very much.
Introduction

- In order for normal zero sodium balance to occur, the amount of sodium that is ingested must equal the amount of sodium that is excreted (mainly in the urine).

- When excess sodium is retained and is not buffered in the interstitial space/lymph, blood pressure increases.

- Sensing the amount of ingested sodium by the stomach/gut is one mechanism by which sodium balance is regulated.
Several gut hormones have been proposed to mediate the natriuresis following an oral sodium load. These gut hormones include gastrin, uroguanylin, and cholecystokinin (CCK), among others.
Gastrin

- Circulating gastrin levels are 10-20-fold higher than CCK levels.

- Of all the gut hormones, gastrin is the one that is taken up to the greatest extent by renal proximal tubules.
Gastrin

- Secreted by G cells in the antrum of the stomach, duodenum, and pancreas.

- Stimulates secretion of gastric acid (HCl) by the parietal cells of the stomach and aids in gastric motility.
Gastrin

- The gastrin receptor is the cholecystokinin B receptor (CCKBR).

- Where is the CCKBR expressed in the kidney?
Sodium-Hydrogen Exchanger Type 3 (NHE3) is expressed in the renal proximal convoluted tubule in mammals, including rodents.
Both NHE3 and CCKBR are expressed in the proximal convoluted tubule in mouse kidney. There is little staining of CCKBR in the glomerulus.
NHE3 and CCKBR colocalize in the proximal convoluted tubule (PCT) in mouse kidney. There is little staining of CCKBR in the glomerulus (G).
Gastric gavage of liquefied chow that increases serum gastrin (~2-fold) also increases absolute (UNaV)

Effect of gastric gavage of liquefied chow on renal function in anesthetized Wistar-Kyoto rats.

*P<0.05 vs Basal,
One-way ANOVA, Tukey’ test
#P<0.05 vs Vehicle, t-test
N=5/group
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Gastric gavage of liquefied chow that increases serum gastrin (~2-fold) also increases absolute (UNaV) and fractional (FNa) sodium excretions.
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Gastric gavage of liquefied chow that increases serum gastrin (~2-fold) also increases absolute (UNaV) and fractional (FNa) sodium excretions and urine flow (UV) that are blocked by the CCKBR antagonist, LY 365-260.

*P<0.05 vs Basal,
One-way ANOVA, Tukey’s test
#P<0.05 vs Vehicle, t-test
N=5/group
A certain amount of sodium in food increases serum gastrin levels (30’ Post-ingestion) in anesthetized salt-resistant (BALB/c) mice

*vs others, One-way ANOVA, Fisher’s LSD test, n=4-6/group
Sodium, independent of food, can increase serum gastrin levels (30’ post-ingestion) in BALB/c mice (Xiaoliang Jiang and Zhiwei Yang)

#P<0.05 vs. other groups,
*P<0.05 vs. distilled H₂O and 0.56% saline, one-way ANOVA, Holm-Sidak test, n=4-6/group
The presence of food increases the ability of sodium to increase serum gastrin levels (30’ post-ingestion) in BALB/c mice (Xiaoliang Jiang and Zhiwei Yang)

#P<0.05 vs. other groups, *P<0.05 vs. distilled H₂O and 0.56% saline, one-way ANOVA, Holm-Sidak test, n=4-6/group
The expression of gastrin is increased in mouse stomach exposed to NaCl ex vivo (30 min)

*P<0.05 vs. Distilled H$_2$O, #P<0.05 vs. others, n=3/group, one-way ANOVA, Holm-Sidak test
Sodium, not chloride or osmolality, is responsible for the increase in the expression of gastrin in mouse stomach exposed to NaCl \textit{ex vivo} (30 min)

*P<0.01 vs. Choline chloride or mannitol, n=3/group, one-way ANOVA, Holm-Sidak test
Expression of gastrin in stomach G cells of \textit{Gast}^{+/+} and \textit{Gast}^{-/-} BALB/c mice
Systolic blood pressure of conscious \textit{Gast}^{-/-} mice is decreased by low sodium (<0.04%) diet and increased by normal sodium (NS) (0.8%) and high (6%) NaCl diet.

\*P<0.05 vs. <0.04% NaCl and \textit{Gast}^{+/+}

\textbf{Gast}^{-/-} \hspace{1cm} n=3-4

\textbf{Gast}^{+/+} \hspace{1cm} n=3-4
Systolic Blood Pressure (BP) (under anesthesia) is increased in Cckbr⁻⁻ mice

*P<0.05 vs Cckbr⁻⁻ or Cckbr⁺⁺ + vehicle, t-test, n=5/group
Systolic Blood Pressure (BP) (under anesthesia) is increased in \(Cckbr^{-/-}\) mice and \(Cckbr^{+/+}\) mice treated with a CCKBR antagonist (YF476).

\*P<0.05 vs \(Cckbr^{-/-}\) or \(Cckbr^{+/+}\) + vehicle, t-test, n=5/group
What causes the increase in blood pressure in $Gast^{-/-}$ mice?
Renal Sodium-Hydrogen Exchanger type 3 (NHE3), Sodium-Potassium-2Chloride Co-transporter (NKCC2), Epithelial Sodium Channels (ENaCs) and Sodium-Potassium ATPase (NKA) are increased in $Gast^{-/-}$ mice.

Data are $M \pm SE$, % of $Gast^{+/-}$ mice, corrected by actin

$P<0.05$, vs. $Gast^{+/-}$ mice, Student’s t-test
The interpretation of the effects of germline deletion of the gene of interest is confounded by compensatory mechanisms during development unless the gene deletion is inducible or organ-specific.
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To determine the role of gastrin released by the stomach and duodenum on sodium excretion after a meal with sodium, we devised a method to silence gastrin secretion by infusing *Gast* siRNA into the celiac artery.
Arterial supply to the stomach and duodenum

Gastroepiploic
Expression of gastrin in the mucosa of the stomach of BALB/c mouse infused with mock siRNA via the celiac artery.
Expression of gastrin is markedly decreased in the stomach of BALB/c mouse infused with *Gast* siRNA via the celiac artery.
The natriuretic response to an oral Na\textsuperscript{+} load (0.84 mmol/kg) is impaired in \textit{Gast\textsuperscript{-/-}} mice and \textit{Gast} siRNA-treated \textit{Gast\textsuperscript{+/+}} mice.
Systolic Blood Pressure (BP) (under anesthesia) is increased in *Cckbr*−/− mice and *Cckbr*+/+ mice treated with a CCKBR antagonist (YF476) and *Gast*+/+ mice treated with *Gast* siRNA infused into the celiac artery.

*P<0.05 vs *Cckbr*−/−, *Cckbr*+/+ + vehicle, or *Gast*+/+ + Mock, t-test, n=5/group

Gast mRNA (stomach)
The sodium-mediated increase in gastrin secretion by the stomach may involve sodium channels.
Sodium channel siRNA (via celiac artery) decreases serum gastrin levels in mice fed normal or high salt (4%NaCl) diet

*S*P* < 0.01 vs. other groups, one-way ANOVA, N=3-4
Sodium channel siRNA (via celiac artery x 3 days) increases systolic blood pressure (BP) in mice fed normal salt (NS, 0.8%NaCl) and high salt (HS, 4% NaCl) diets but not low salt (LS, 0.04% NaCl) diet.

*S*P<0.05, vs. Mock, one-way ANOVA, N=4-7
We also tested the hypothesis that the sodium-mediated increase in gastrin secretion by the stomach may involve sodium channels in gastric carcinoma (N87) cells incubated (30 min) in media with varying sodium concentrations.
Intracellular sodium (sodium green staining) accumulation is increased in N87 (gastric carcinoma) cells incubated (30 min) in media with increased sodium concentration.

- 90 mM extracellular NaCl
- 143 mM extracellular NaCl
- 170 mM extracellular NaCl
Expression of gastrin (GAST) in N87 (gastric carcinoma) cells is greater in cells exposed to 143 than 90 mM NaCl for 4 hours.

*P<0.05 vs. 90 mM NaCl, t-test
n=4/group
Cultured G cells isolated from mouse stomach do not retain their normal phenotype.

Drs. Gildea, Xu, and Felder were able to isolate and culture G cells from human stomach that retain their phenotype.
An increase in extracellular sodium concentration increases sodium channel activity (measured by patch clamp) in human G cells.

Summary

- Tag 1: 0.035 min, 145 mM Na+
- Tag 2: 1.102 min, 170 mM
- Tag 3: 3.108 min, 145 mM wash out

n=6/group, *P<0.05 vs both 140 concentrations, repeat measures of ANOVA, Tukey's Multiple Comparison Test
Gastrin is important in the excretion of a sodium load. However, there are other natriuretic hormones that are increased after a sodium load.
Dopamine

- Dopamine produced by the kidney is also critically involved in the excretion of a sodium load.

- Do gastrin and dopamine interact?
The Proximal tubular Dopaminergic System

Dopamine receptors

Renal Dopamine Synthesis

Dopamine

AADC

Renal Proximal Tubule

Basolateral membrane

Renal Dopamine Synthesis

LAT-2

L-DOPA

rBAT

Brush Border

Dopamine receptors

Dopamine
The Proximal tubular Dopaminergic System

Renal Dopamine Synthesis and Dopamine Egress into the Lumen (increased by salt loading)

- Dopamine receptors
- AADC
- Renal Proximal Tubule
- Basolateral membrane
- L-DOPA
- LAT-2
The Proximal tubular Dopaminergic System

Dopamine receptors

HVA

DOPAC

3 MT

Renal Dopamine Degradation (decreased by salt loading)

Basolateral membrane

Renal Proximal Tubule

Brush Border

Dopamine

COMT

MAO-A/C
Dopamine produced in renal tubules (high nM) from L-DOPA is not converted to norepinephrine.

Dopamine exerts its renal autocrine/paracrine function by regulating renal tubular transport mechanisms.

Circulating concentrations of dopamine (pM) are too low to stimulate its own receptors → affinity (nM).

Dopamine administered to increase blood pressure acts on non-dopaminergic receptors (β and α-adrenergic receptors).
**Dopamine and Renal Function**

- Inhibits multiple sodium transporters/pumps – dopamine is multifunctional

- Multiple sites of action - proximal tubule, thick ascending limb, distal convoluted tubule, collecting duct

Classification of Dopamine Receptors

Family

$D_1$-like

$D_2$-like
### Classification of Dopamine Receptors

<table>
<thead>
<tr>
<th>Family</th>
<th>Subtype</th>
<th>Effector</th>
<th>Structure (amino acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D₁-like</td>
<td>D₁ (chr 5)</td>
<td>$G_S$</td>
<td>466</td>
</tr>
<tr>
<td></td>
<td>D₅ (chr 4)</td>
<td>$G_S$</td>
<td>477</td>
</tr>
<tr>
<td>D₂-like</td>
<td>D₂ (chr 11)</td>
<td>$G_{i/O}$</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>D₃ (chr 3)</td>
<td>$G_{i/O}$</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>D₄ (chr 11)</td>
<td>$G_{i/O}$</td>
<td>387</td>
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</table>
Stimulation of D₁ dopamine receptors decreases the amount of NaCl transported from the proximal tubule into the peritubular capillary and into the blood stream.

More NaCl is excreted in the urine preventing the accumulation of NaCl in the body.

When moderate amounts of NaCl are ingested, the intrarenal dopaminergic system is responsible for more than 50% of urinary sodium excretion.
Low concentrations of systemically administered Gastrin 17 (100 pmol/Kg/min) and D₁-like receptor agonist, Fenoldopam (Fen, 1 µg/Kg/min) synergistically increase fractional sodium excretion in WKY rats.

*P<0.01 vs. others
One-way ANOVA, Newman-Keuls test n=3/group
The natriuretic effect of a D<sub>1</sub>-like receptor agonist, Fenoldopam, can be blocked not only by a D<sub>1</sub>-like receptor antagonist, SCH23390, but also by a gastrin receptor (CCKBR) antagonist, CI-988.

The natriuretic effect of gastrin is blocked by D$_1$-like receptor antagonist (SCH23390) and also by Gastrin receptor (CCKBR) antagonist (CI-988) (Chen Y and Zeng C, et al. Hypertension 2013;62:927-33)
Hypothesis

- The increase in sodium excretion after a meal is due to the presence of a critical amount of sodium in the food which increases gastrin secretion and, in turn, increases dopamine production by the kidney.
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- Dopamine acting on renal dopamine receptors and gastrin on renal CCKB receptors then inhibit sodium transport, resulting in a natriuresis, independent of any increase in blood pressure, a gastro-renal axis.
The GUT enables the kidney to increase sodium chloride excretion in response to an increase in sodium intake by increasing the secretion of enterokines.
After ingesting too much salt, the stomach begs the kidney: please rectify my dietary indiscretion!

Taste (Salt) Receptors?

Gastro-renal Axis
Acute Oral Sodium Load:
Gastrin and Dopamine Interaction

Hypothesis

Sodium Intake

G-cells

↑ gastrin

↑ L-DOPA Dopamine
Hypothesis

**Acute Oral Sodium Load:**
Gastrin and Dopamine Interaction

- **Sodium Intake**
- **G-cells**
- **gastrin**
- **L-DOPA Dopamine**
- **Circulating L-DOPA**
Acute Oral Sodium Load: Gastrin and Dopamine Interaction

Hypothesis

Sodium Intake

G-cells

↑ gastrin

↑ L-DOPA Dopamine

Circulating L-DOPA

↑ Dopamine

↑ Dopamine receptors

↑ UNaV
Colocalization of G cells and tyrosine hydroxylase (TH) in mouse stomach mucosa
Colocalization of gastrin and DOPA decarboxylase (DDC) in mouse stomach

DDC (rabbit, 1:100)

Gastrin (goat, 1:100)

Merge
Ingestion of sodium increases dopamine production in the stomach

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stomach</th>
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</tr>
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<td>DOPA (pmol/mg tissue)</td>
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*P<0.05 vs. dH₂O; N=3/group
Ingestion of sodium increases dopamine production in the stomach and L-DOPA levels in the kidney

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*P<0.05 vs dH₂O; N=3/group
Mechanisms by which gastrin may increase renal L-DOPA levels

- Increased production of L-DOPA by stimulating tyrosine hydroxylase (TH) activity
  → unlikely, TH is not expressed in renal tubules

- Increased conversion of L-DOPA to dopamine by stimulating DOPA decarboxylase activity - has not been reported

- Increased renal uptake of L-DOPA
  → most likely mechanism
Gastrin enhances the uptake of L-DOPA in renal proximal tubule cells resulting in increased renal dopamine

*P<0.05 vs all others Fisher’s LSD test, n=3-4/group
Ingestion of sodium increases dopamine production in the stomach but decreases it in the kidney

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*P<0.05 vs dH₂O; N=3/group
Urinary dopamine increases after a NaCl gavage in mice

*P<0.05 vs. distilled H$_2$O, One-way ANOVA, Holm-Sidak test

*P<0.05 vs. all others, Fisher’s LSD test, n=3-4/group

*P<0.05 vs. distilled H$_2$O, One-way ANOVA, Holm-Sidak test
Summary

Effect of food and sodium on gastrin secretion and sodium excretion:

- Food intake, with some amount of sodium, increases gastrin secretion.
- Sodium, independent of food, increases gastrin secretion.
- Food intake, with some amount of sodium, increases gastrin secretion to a greater extent than sodium intake without food.
Effect of gastrin and dopamine on sodium excretion:

- Gastrin and dopamine increase sodium excretion following food and sodium intake.

- Gastrin receptors (CCKBR) and D₁-like receptors may synergize to increase renal sodium excretion.
Conclusion

Gastrin may be the GUT hormone, aided by renal dopamine, that signals the kidney to excrete sodium after ingesting food with a critical amount of sodium.
If I am not for myself, who will be for me? If I am only for myself, what am I?
And if not now, when? (Hillel the Elder, Pirkei Avot, 1:14)

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• Zheng Wang
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• Xiaoxu Zheng
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• David R. Sibley
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• Tsuyoshi Watanabe
• Matthew Weir
• William J. Welch
• Christopher S. Wilcox
• Scott M. Williams
• Dan Wang
• Lee-jun Wong
• Hiyoshi Yamaguchi
• Hirohide Yokokawa
• Minoru Yoneda

Funding: NIH (NHLBI, NIDDK, NCRR), NKF, AHA
Annabel Lee

By Edgar Allan Poe

It was many and many a year ago,
In a kingdom by the sea,
That a maiden there lived whom you may know
By the name of Annabel Lee;
And this maiden she lived with no other thought
Than to love and be loved by me.
Annabel Lee

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Sancho Panza

Don Quijote

Other awardees of the Excellence Award for Hypertension Research

Pedro A. Jose
Thank you