New Insights into the Physiological Functions of Renal (Pro)Renin Receptor

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Department of Internal Medicine, University of Utah;
Institute of Hypertension, Sun Yat-sen University
Furin or ADAM19
M8.9 (V-H+-ATPase; ATP6ap2)

sPRR (binding to prorenin/renin)

PRR (350 AA, 37-43 kDa)

Signal peptide
Extracellular
Cleavage site
TM
Cytoplasmic

Ludwig J et al. JBC 1998
Nguyen G et al. JCI 2002

M8.9 (V-H+-ATPase; ATP6ap2)
Alignment of PRR amino acid sequences

**Consenus**

- **Signal peptide**
- **Extracellular domain**
- **Transmembrane domain**
- **Intracellular domain**
- **Furin site**

**Alignment**

- **H. sapiens / f-350**
- **F. abelis / f-350**
- **F. juglandis / f-350**
- **N. musculus / f-350**
- **X. laevis / f-348**
- **C. elegans / f-324**

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- **H. sapiens / f-350**
- **F. abelis / f-350**
- **F. juglandis / f-350**
- **N. musculus / f-350**
- **X. laevis / f-348**
- **C. elegans / f-324**

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- **H. sapiens / f-350**
- **F. abelis / f-350**
- **F. juglandis / f-350**
- **N. musculus / f-350**
- **X. laevis / f-348**
- **C. elegans / f-324**

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- **H. sapiens / f-350**
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- **F. juglandis / f-350**
- **N. musculus / f-350**
- **X. laevis / f-348**
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- **H. sapiens / f-350**
- **F. abelis / f-350**
- **F. juglandis / f-350**
- **N. musculus / f-350**
- **X. laevis / f-348**
- **C. elegans / f-324**

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**Legend**

- : Signal peptide
- : Extracellular domain
- : Transmembrane domain
- : Intracellular domain
- : Furin site
- : Intracellular domain
(Pro)Renin Receptor (ATP6AP2) Signaling via Wnt/β-catenin in Xenopus

Cruciat CM et al. Science 2010
Inhibition of PRR Causes Developmental Abnormality in Xenopus

Cruciat CM et al. Science 2010
Lethal Phenotype in Various PRR KO Mice

- Conventional PRR KO
- Cardiomyocyte PRR KO
- Podocyte PRR KO
- CD PRR KO (Hoxb7 Cre)
Limited Knowledge on Pleiotropic Functions of PRR
In Vitro Evidence for Renin Regulatory Property of PRR

Nguyen G et al. JCI 2002
Consecutive rat kidney sections stained for either anion exchanger 1 (AE1), which is expressed on the basolateral border of A-ICs (A, thin arrow) or (P)RR (B).

Advani et al. Hypertension. 2009;54:261-269
Summary of Renal Prostaglandin Research

- Renin secretion
- AngII action
- Water transport
- Na transport
- Renal blood flow
- Renal medullary blood flow
- Postnatal kidney development

**Pathways:**
- COX-2
- PGES
- PGE2
- EP1-4
COX-2/PGE2 Pathway Mediates AngII-Induced Renin Response and PRR Expression in CD Cells

Prostaglandin E-Prostanoid Receptor Mediates Angiotensin II–Induced (Pro)Renin Receptor Expression in the Rat Renal Medulla

Prorenin/ Renin

Intrarenal RAS

BP

AngII

COX-2/EP4

PRR

Key Words: prorenin, renin, angiotensin II, angiotensin

In recent months, there has been growing interest about the local renin–angiotensin system (RAS) in the context of hypertension, including the kidney. In the setting of renal disease, angiotensin II is expressed in the proximal tubule and is the contributing mechanism and cortical and medullary collecting ducts (CCD), forming the afferent limbs of the Afferent RAS. In response to angiotensin II, the intrarenal RAS is activated as described below.

The specific cell type or the mechanism by which angiotensin II is produced and expressed in the CCD will be discussed in the following sections. Several lines of evidence demonstrate a major role for intrarenal RAS in blood pressure regulation. For example, intrarenal angiotensin II has been shown to be produced and expressed in the renal interstitium. Intrarenal angiotensin II is produced by the renin–angiotensin system, which is activated by angiotensin II or other stimuli. The intrarenal RAS not only regulates blood pressure but also modulates renal function, including renal blood flow and glomerular filtration rate. Therefore, the intrarenal RAS is a critical component of the renin–angiotensin system, and understanding its function is crucial for the development of effective therapeutic strategies for hypertension and other renal diseases.

Angiotensin II is produced in the kidneys by the renin–angiotensin system, which is activated by angiotensin II or other stimuli. The intrarenal RAS is produced by the renin–angiotensin system, which is activated by angiotensin II or other stimuli. The intrarenal RAS not only regulates blood pressure but also modulates renal function, including renal blood flow and glomerular filtration rate. Therefore, the intrarenal RAS is a critical component of the renin–angiotensin system, and understanding its function is crucial for the development of effective therapeutic strategies for hypertension and other renal diseases.
MAP in Rats Receiving Intramedullary or Intravenous Infusion of PRO20

Effect of Intramedullary Infusion of a PRR Blocker on AngII-Induced Urinary Renin Activity

Wang F et al. BMC Medicine 2015
Effect of PRR Antagonism on Renal $\alpha$-ENaC Expression in AngII-Induced Hypertension
Antidiuretic Action of Collecting Duct (Pro)Renin Receptor Downstream of Vasopressin and PGE$_2$ Receptor EP$_4$

Fei Wang,*† Xiaohan Lu,† Kexin Peng,† Hui Fang,* Li Zhou,* Jiahui Su,* Adam Nau,† Kevin Yang,† Atsuhiro Ichihara,‡ Aihua Lu,* Shu-Feng Zhou,* and Tianxin Yang*†

*Institute of Hypertension, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China; †Department of Internal Medicine, University of Utah and Veterans Affairs Medical Center, Salt Lake City, Utah; ‡Department of Medicine II, Endocrinology and Hypertension, Tokyo Women’s Medical University, Tokyo, Japan; and §Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, Florida

Dehydration

COX-2/EP4

Prorenin/Renin

PRR

AQP2

Urine concentrating capability
Aqp2-Expressing Cells Give Rise to Renal Intercalated Cells

Hongyu Wu,* Lihe Chen,† Qiaoling Zhou,‡ Xi Zhang,* Stefan Berger,§ Jiong Bi,* Dorothy E. Lewis,*† Yang Xia,†† and Wenzheng Zhang*†

*Department of Internal Medicine and †Graduate School of Biomedical Sciences and ‡Department of Biochemistry and Molecular Biology, The University of Texas Health Science Center at Houston, Houston, Texas; §Department of Internal Medicine, Xiangya Hospital, Central South University, Changsha, Hunan, China; and ‡German Cancer Research Center, Division Molecular Biology of the Cell I, Heidelberg, Germany
Double Transgenic Mice for AQP2-Cre and ROSA26-YFP
DNA Recombination in CD PRR KO Mice

Skin

Brain

Renal cortex

Renal medulla

Brain

Floxed KO

Floxed KO

Floxed KO

Floxed KO

Floxed KO

Floxed KO

Floxed KO

Floxed KO

Floxed KO

PRR

β-actin
MAP in CD PRR KO Mice after AngII Infusion at 300 ng/kg/min

Peng K et al. AJP-Renal 2016
Downregulation of Renal Medullary α–ENaC in CD PRR KO mice after AngII Infusion

**A**

<table>
<thead>
<tr>
<th></th>
<th>Floxed</th>
<th>KO</th>
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<tbody>
<tr>
<td></td>
<td>CTR</td>
<td>AngI</td>
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<tr>
<td>α-ENaC</td>
<td></td>
<td></td>
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<tr>
<td>β-ENaC</td>
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<td></td>
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<tr>
<td>γ-ENaC</td>
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**B**

- Basal
- Ang II

<table>
<thead>
<tr>
<th>β-ENaC mRNA/GAPDH</th>
<th>Floxed</th>
<th>KO</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Ang II</td>
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<tr>
<td>α-ENaC</td>
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<tr>
<td>β-ENaC</td>
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<table>
<thead>
<tr>
<th>Basal</th>
<th>Ang II</th>
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<tr>
<td>1.6</td>
<td>2.4</td>
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**C**

<table>
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<th>β-ENaC mRNA/GAPDH</th>
<th>Floxed</th>
<th>KO</th>
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<tr>
<td></td>
<td>Basal</td>
<td>Ang II</td>
</tr>
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</table>

| 1.2 |
| 1.6 |

**D**

<table>
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<th>γ-ENaC mRNA/GAPDH</th>
<th>Floxed</th>
<th>KO</th>
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<tr>
<td></td>
<td>Basal</td>
<td>Ang II</td>
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| 0.8 |
| 1.2 |

Downregulation of α–ENaC in CD PRR KO mice after AngII Infusion
shRNA-mediated knockdown of PRR reduces renal medullary α-ENaC expression in rats

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<th></th>
<th>Vehicle</th>
<th>scramble</th>
<th>shRNA</th>
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<tbody>
<tr>
<td>α-ENaC</td>
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</tr>
<tr>
<td>β-Actin</td>
<td></td>
<td></td>
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Quadri S et al. J Hypertens 2016
Measurement of sodium transport across the monolayer of renal epithelial cells
Prorenin Increases Sodium Transport in mpkCCD Cells via PRR

Lu X AJP-Renal 2015
Prorenin activation of PRR stimulates AQP2 expression in primary rat IMCD cells

Wang F et al. JASN 2016
Lu X et al. *PNAS* 2016

Activation of RAAS

Dehydration

Prorenin

COX-2-EP4

PRR

Principle Cell

Intercalated Cell

Luminal

ENaC

AQP2

Na+

H₂O
Immunostaining with Antibodies Recognizing Different PRR Domains

AQP2

Anti-PRR-N antibody

Anti-PRR-N antibody

Anti-PRR-C antibody

PRR

A

B

C

D

E

F

G

H

I

J

K

L

PPR (350 AA, 37-43 kDa)

Anti-PRR-N antibody

Anti-PRR-C antibody

Furin or ADAM19

Merge

Anti-PRR-N antibody

Anti-PRR-N antibody + sPRR-His

Absence of primary antibody

Lu et al. *PNAS* 2016
Effect of sPRR-His on Na⁺ Transport in Cultured mpkCCD Cells

Lu et al. PNAS 2016
The Recombinant sPRR Upregulates ENaC Expression in mpkCCD Cells

α-ENaC
CTR sPRR-His
95 kDa

β-Actin
CTR sPRR-His
43 kDa

ENaC densitometry/β-actin

CTR
sPRR-His

*
Effect of sPRR-His on AQP2 Expression in Cultured IMCD Cells

Promoter activity assay

Luminescence (RLU/µg protein)

CTR  sPRR-His

35~45 kDa

28 kDa

43 kDa

Lu et al. PNAS In press 2016
SPRING-Based Prediction of Proteins That May Interact with sPRR
Interaction of sPRR and Frizzled8 and Immunostaining of Frizzled8
OMP and ICG Blocked sPRR-His Induced β-Catenin Signaling Activation in mpkCCD cells

Luminescence assay for LCF/TCF activity

- CTR
- sPRR-His
- sPRR-His + OMP

OMP: Frizzled-8 inhibitor

Luminescence assay for LCF/TCF activity

- CTR
- sPRR-His
- sPRR-His + ICG

ICG: β-catenin inhibitor
OMP Blocked sPRR-His Induced ENaC Activation in mpkCCD Cells

OMP: Frizzled-8 inhibitor
Normal Renal Histology in CD β-Catenin KO Mice

Floxed  CD β-catenin KO
Blunted Hypertensive Response to Aldosterone/Salt in CD β-Catenin KO Mice

![Graph showing MAP (mmHg) over days of treatment for Floxed and CD β-catenin KO mice. The graph illustrates a blunted hypertensive response to Aldosterone/Salt treatment.]
Effect of Inhibition of Wnt/β-Catenin Pathway on AngII-Induced Hypertension in Mice
Restoration of AngII-induced Hypertensive Response by sPRR-His in CD PRR KO Mice
sPRR-His Attenuates DI Induced by V2R Antagonism in Mice

Lu X et al. PNAS 2016
sPRR-His Attenuates DI in PRR KO Mice

CD PRR KO mice

Urine volume (ml/24h)

Floxed KO KO + sPRR-His

Urine osmolality (mOsm/kg.H2O)

Floxed KO KO + sPRR-His

Nephron PRR KO mice

Urine volume (ml/24h)

KO KO + sPRR-His

Urine osmolality (mOsm/kg.H2O)

KO KO + sPRR-His

* * * *

KO KO + sPRR-His

Basal Day 4 Day 7 Day 10

Basal Day 4 Day 7 Day 10
AngII  Dehydration  Fructose/Salt

COX-2/EP4

Furin  Prorenin

VSMC  Intercalated cell

Endocrine/Paracrine  Paracrine

Na+  β-Catenin  LRP5/6

FZD8

H2O  AQP2  ENaC

PRR

Low plasma volume (shock, diabetes insipidus, etc.)

Activation (sPRR-His)

High plasma volume (hypertension, cardiac failure, cirrhosis, edema, etc.)

Inhibition (PRO20)

Na and water reabsorption

Plasma volume and blood pressure

AQP2  ENaC

Principal cell

Fructose/Salt  AngII  Dehydration
ACKNOWLEDGEMENTS

Yang Lab

Fei Wang
Xiaohan Lu
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Kexin Peng
Changjiang Zou
Long Zhao
Kevin Yang
Adam Nau

Collaborators

Yumei Feng
Atsuhiro Ichihara
Donald E. Kohan
Nirupama Ramkumar
Jan-Åke Gustafsson
OncoMed Pharmaceuticals

Funding:

RO-1 DK066592
RO-1 DK055119
VA Merit Review
VA Research Career Scientist
NSFC
<table>
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<th>ACKNOWLEDGEMENTS</th>
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<td><strong>Yang Lab</strong></td>
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<td>Fei Wang</td>
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<td>Chuanming Xu</td>
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<td>Kexin Peng</td>
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<td>Long Zhao</td>
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OMP and ICG Blocked sPRR-induced α-ENaC Protein Expression in mpkCCD Cells

**Graphs:**
- **left graph:**
  - CTR, sPRR-His, sPRR-His+OMP
  - α-ENaC: 95 kDa
  - β-Actin: 43 kDa

- **right graph:**
  - CTR, sPRR-His, sPRR-His+ICG
  - α-ENaC: 95 kDa
  - β-Actin: 43 kDa

**Bar charts:**
- ENaC densitometry/β-actin

**Legend:**
- *: Significant difference compared to CTR
- #: Significant difference compared to sPRR-His
Protein Expression of PRR in Drosophila and Mouse Kidney

<table>
<thead>
<tr>
<th>Protein</th>
<th>Species</th>
<th>M.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>fPRR</td>
<td>Drosophila</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mouse kidney</td>
<td>37 kDa</td>
</tr>
</tbody>
</table>
Canonical AVP Signal Transduction Pathway

Luminal

H₂O
H₂O
H₂O
H₂O
CD

AQP2

Acute regulation

PKA

cAMP

AC

V2R

AVP
Dehydration

Basolateral

H₂O
H₂O
H₂O

p
p

Chronic regulation

CREB

AQP₂

Principal Cell
Outline

- General function of CD PRR in regulation of water transport
- sPRR signaling in water regulation
- In vivo antidiuretic action of sPRR in various DI models
sPRR-His Increased Urinary sPRR Excretion

![Graph showing increased urinary sPRR excretion](image)

- Floxed
- Floxed CD PRR KO
- Floxed CD PRR KO + sPRR-His
- CD PRR KO
- Neph PRR KO
- Neph PRR KO + sPRR-His

* Indicates significant difference
# Indicates significant difference
sPRR-His Attenuates DI in PRR KO Mice

**CD PRR KO mice**

- Urine volume (ml/24h)
- Floxed, KO, KO + sPRR-His

- Urine osmolality (mOsm/kg H2O)
- Floxed, KO, KO + sPRR-His

**Nephron PRR KO mice**

- Urine volume (ml/24h)
- Basal, Day 4, Day 7, Day 10
- KO, KO + sPRR-His

- Urine osmolality (mOsm/kg H2O)
- Basal, Day 4, Day 7, Day 10
- KO, KO + sPRR-His
sPRR-His Increases AQP2 Expression

**CD PRR KO mice**

- KO
- KO + sPRR-His

<table>
<thead>
<tr>
<th>40 kDa</th>
<th>35~45 kDa</th>
<th>29 kDa</th>
<th>43 kDa</th>
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**AQP2**

**β-Actin**

**Nephron PRR KO mice**

- KO
- KO + sPRR-His

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**AQP2**

**β-Actin**
sPRR-His Restores AVP Sensitivity

Floxed CD PRR KO CD PRR KO + sPRR-His

Ratio of the urinary osmolality response to acute VP treatment

Floxed Nephron PRR KO Nephron PRR KO + sPRR-His

Ratio of the urinary osmolality response to acute VP treatment
No Effect of sPRR-His on NKCC2 expression

<table>
<thead>
<tr>
<th>Floxed</th>
<th>Nephron PRR KO</th>
<th>Nephron PRR KO</th>
<th>Nephron PRR KO + sPRR-His</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKCC2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td></td>
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</tbody>
</table>

![Western Blot Images]

**Graph:**

- **y-axis:** NKCC2 mRNA/GAPDH
- **x-axis:** CTR, Neph PRR KO, Neph PRR KO + sPRR-His

- **CTR:** 1.2
- **Neph PRR KO:** 0.3
- **Neph PRR KO + sPRR-His:** 0.3

*Significant difference compared to CTR.
### Effect of sPRR-His on Autophagy Markers in PRR KO Mice

<table>
<thead>
<tr>
<th>Renal outer medulla</th>
<th>Floxed Nephron PRR KO</th>
<th>Nephrorn PRR KO</th>
<th>Nephrorn PRR KO + sPRR-His</th>
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</thead>
<tbody>
<tr>
<td>p62</td>
<td><img src="image1.png" alt="Image of p62" /></td>
<td><img src="image2.png" alt="Image of p62" /></td>
<td><img src="image3.png" alt="Image of p62" /></td>
</tr>
<tr>
<td>LC3b</td>
<td><img src="image1.png" alt="Image of LC3b" /></td>
<td><img src="image2.png" alt="Image of LC3b" /></td>
<td><img src="image3.png" alt="Image of LC3b" /></td>
</tr>
</tbody>
</table>

- **p62**: 83 kDa
- **LC3b**: 17 kDa
Autophagosome Markers in the Kidney of CD PRR KO Mice

- **p62**: Floxed (83 kDa) vs. CD PRR KO (17 kDa)
- **LC3b**: Floxed (17 kDa) vs. CD PRR KO (17 kDa)
- **β-actin**: Floxed (43 kDa) vs. CD PRR KO (43 kDa)
EM Analysis of the CD in CD PRR KO Mice

CD for CD PRR KO

A

B
EM Analysis of the TAL in CD PRR KO Mice

TAL for CD PRR KO

10 µm 10 µm
AVP

Fluid volume/BP

PRR

sPRR

V2R-AQP2 axis

Water reabsorption

V1a

Vascular constriction

Fluid volume/BP
COX-2/EP4 Pathway Mediates AngII-Induced Renin Response and PRR Expression in CD Cells

Wang et al. *AJP-Renal* 2014; Wang et al. *Hypertension* 2014; Gonzalez et al. *AJP-Renal* 2014
BP Lowering Effect of ICGP in DOCA/Salt and ICG-001
Immunoblotting of PRR in Renal Inner Medulla of Rats Treated with AngII alone or in Combination with ONO

CTR          Ang II          Ang II + ONO

PRR          43kDa          43kDa

β-Actin      42 kDa         42 kDa

Wang et al. *Hypertension* 2014
Urinary and Renal Medullary Renin Activity

Renal inner medullary renin activity (Ang I ng/hr/µg)

CTR  |  AngII  |  AngII + ONO

Urine renin activity (Ang I ng/24hr)

CTR  |  AngII  |  AngII + ONO

$p<0.05$
Mean Arterial Pressure (MAP) in Rats over 7 Days of Ang II Infusion with or without EP4 Antagonism

Wang et al. *Hypertension* 2014
MAP in Rats Receiving Intramedullary or Intravenous Infusion of PRO20

Effect of Intramedullary Infusion of PRO20 on AngII-Induced Renal Medullary Renin Levels

Effect of PRR Antagonism on Renal α-ENaC Expression in AngII-Induced Hypertension
shRNA-mediated knockdown of PRR reduces renal medullary α-ENaC expression in rats

Quadri S et al. J Hypertens 2016
MAP in CD PRR KO mice after AngII Infusion
Reduction of renal medullary $\alpha$-ENaC in CD PRR KO Mice

<table>
<thead>
<tr>
<th></th>
<th>Floxed</th>
<th>CD PRR KO</th>
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<td></td>
<td>CTR</td>
<td>AngII</td>
</tr>
<tr>
<td></td>
<td>CTR</td>
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<tr>
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Measurement of sodium transport across the monolayer of renal epithelial cells
Prorenin Increases Na+ Transport in mpkCCD Cells via PRR

Lu X et al. AJP-Renal 2015
AQP2 Regulation in Primary Rat IMCD Cells in Transwell
Prorenin activation of PRR stimulates AQP2 expression in primary rat IMCD cells

Wang F et al. JASN In press 2016
Structure and Cleavage of (Pro)Renin Receptor (PRR)

PRR (350 AA, 37-43 kDa)

Soluble PRR (28 kDa)

Furin
Immunostaining with Antibodies Recognizing Different PRR Domains

- Anti-PRR-N antibody
  - 200X
  - 400X

- Anti-PRR-C antibody
  - 400X

- Furin or ADAM19

- Anti-PRR-N antibody

- Anti-PRR-C antibody

- Anti-PRR-N antibody + sPRR-His

- Absence of primary antibody

Lu et al. PNAS In press
Collecting duct PRR regulates local renin response and ENaC and thus determines AngII-induced hypertension.

sPRR derived from intercalated cells may act in a paracrine fashion to regulate Na+ and water transport in principal cells.

FZD8/beta-catenin pathway mediates the Na+ and water retaining and prohypertensive actions of CD PRR.

PRO20 may offer a novel intervention for hypertension and renal disease
### ACKNOWLEDGEMENTS

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<th><strong>Yang Lab</strong></th>
<th><strong>Collaborators</strong></th>
<th><strong>Funding:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fei Wang</td>
<td>Yumei Feng</td>
<td>NIH</td>
</tr>
<tr>
<td>Xiaohan Lu</td>
<td>Nebraska Univ.</td>
<td>Dept. of Veterans Affairs</td>
</tr>
<tr>
<td>Chuanming Xu</td>
<td>Atsuhiro Ichihara</td>
<td>AHA</td>
</tr>
<tr>
<td>Kexin Peng</td>
<td>Tokyo Women's</td>
<td>NSFC</td>
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<tr>
<td>Changjiang Zou</td>
<td>Medical Univ.</td>
<td></td>
</tr>
<tr>
<td>Long Zhao</td>
<td>Donald E. Kohan</td>
<td></td>
</tr>
<tr>
<td>Adam Naum</td>
<td>Univ of Utah</td>
<td></td>
</tr>
<tr>
<td>Maicy Downton</td>
<td>OncoMed Pharmaceuticals</td>
<td></td>
</tr>
</tbody>
</table>
In Vitro Evidence for Renin Regulatory Property of PRR

Prorenin

Prosegment

Non-proteolytic

Proteolytic

Conformational change

Angiotensinogen

Prosegment

ERK1/2

Renin

Angl
Fig. 8 ENaC q-PCR

CTR AngII AngII + Comp A

α ENaC mRNA/GAPDH

CTR AngII AngII + Comp A

β ENaC mRNA/GAPDH

CTR AngII AngII + Comp A

γ ENaC mRNA/GAPDH
Fig. 9 ENaC activity

Bar graph:
- CTR
- Aldo
- Aldo + Comp A

Time course graph:
- CTR
- Aldo
- Aldo + Comp A

Axes:
- Y-axis: Amiloride-sensitive ENaC channel activity (µA/cm²)
- X-axis: Time course (0h, 6h, 12h, 24h, Amiloride)
Fig. 10  AQP2-pAQP2

![Bar graph showing urine osmolality for CTR, AngII, and AngII + Comp A](image)

![Image showing Renal medullary AQP2](image)

![Image showing Renal medullary p-AQP2](image)
Fig. 11 Immunostaining of AQP2
Fig. 13 immunostaining of kidney injury
PAS staining
Time Course of AngII Stimulation of PRR Expression in Primary Rat IMCD Cells

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRR</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>β-Actin</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>

PRR: 43 kDa
β-Actin: 42 kDa
Structure and Cleavage of (Pro)Renin Receptor (PRR)

PRR (350 AA, 37-43 kDa)

Soluble PRR (28 kDa)

Furin
ELISA Detection of Medium Soluble (Pro)Renin Receptor

Graph showing solubility of (P)RR in different conditions:
- CTR
- AngII
- AngII + EP4 antagonist

Significance levels:
- $P < 0.01$
- $P < 0.05$
- $P > 0.05$
Effect of EP4 Agonist on PRR Expression

CTR CAY10598

PRR

β-Actin

43 kDa

42 kDa

Wang et al. *Hypertension* 2014
Ang II Stimulates COX-2 Expression in Primary Rat IMCD Cells

Control  | Ang II (4 hr) | Ang II (8 hr) | Ang II (12 hr)

COX-2

43 kDa

Vehicle  | Ang II (4hr) | Ang II (8hr) | Ang II (12hr)

P<0.05
PRR Protein Expression in IMCD Cells after 12 Hours of AngII Treatment with or without NS-398

Vehicle  | Ang II  | Ang II + NS-398
---|---|---
PRR | Ang II | Ang II + NS-398

43 kDa

P<0.05

P<0.01
Medium Renin Activity

![Graph showing Ang I ng/ml/hr for Vehicle, ANG II, and ANG II + NS-398. The graph indicates that ANG II has a significantly higher Ang I ng/ml/hr compared to Vehicle and ANG II + NS-398, both with P<0.05.](image-url)
Effect of Exogenous PGE2 on PRR Expression

Vehicle

PGE2

PRR

43 kDa

P<0.01
Exogenous PGE2 Reverses the Effect of NS-398
**Prorenin prosegment**

Prorenin: LPTDTASFGRILLKKMPSVREILEERGVMTRISA EWGEFI KK – Renin

HRP: NH$_2$RILLKKMPSV-COOH

**B**

Relative intensity (%)

<table>
<thead>
<tr>
<th>Relative intensity (%)</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1185.7</td>
</tr>
</tbody>
</table>

Ichihara et al. *JCI* 2004
(Pro)Renin Receptor Peptide Inhibitor “Handle-Region” Peptide Does Not Affect Hypertensive Nephrosclerosis in Goldblatt Rats

Dominik N. Muller, Bernd Klanke, Sandra Feldt, Nada Cordasic, Andrea Hartner, Roland E. Schmieder, Friedrich C. Luft, Karl F. Hilgers

Abstract—The (pro)renin receptor [(P)RR], a new component the renin-angiotensin system, was cloned recently. The (P)RR promotes direct mitogen-activated protein kinase signaling and nonproteolytic prorenin activation. We investigated the role of a (P)RR blocker, a peptide consisting of 10 amino acids from the prorenin prosegment called the “handle-region” peptide (HRP), on target organ damage in renovascular hypertensive 2-kidney, 1-clip (2K1C) rats. Vehicle-treated 2K1C rats were compared with HRP-treated 2K1C rats (3.5 μg/kg per day) and sham-operated controls. Vehicle-treated 2K1C rats developed hypertension (186±17 mm Hg), cardiac hypertrophy (3.16±0.16 mg/g), renal inflammation, fibrosis, vascular, and tubular damage. Chronic HRP treatment did not affect blood pressure (194±15 mm Hg), cardiac hypertrophy (2.97±0.11 mg/g), or renal damage. Furthermore, we investigated the renal renin and (P)RR expression. The clipped kidney of 2K1C and HRP-treated 2K1C rats showed a higher renin expression and juxtaglomerular index compared with sham-operated kidneys. The unclipped kidney showed suppressed renin expression. In contrast, (P)RR mRNA expression was not altered in any group. Plasma renin activity and aldosterone were increased in 2K1C rats compared with sham controls. HRP-treated 2K1C rats tended to lower plasma renin activity but showed similar aldosterone levels as vehicle-treated 2K1C rats. Our results indicate that blockade of the (P)RR with HRP does not improve target organ damage in renovascular hypertensive rats. (Hypertension. 2008;51:676-681.)

Key Words: renin ■ (pro)renin receptor ■ HRP ■ target organ damage ■ angiotensin ■ renovascular hypertension
The Putative (Pro)renin Receptor Blocker HRP Fails to Prevent (Pro)renin Signaling

Sandra Feldt,* Ulrike Maschke,† Ralf Dechend,* Friedrich C. Luft,*† and Dominik N. Muller*†

*Medical Faculty of the Charité, Experimental and Clinical Research Center, Franz Volhard Clinic, and HELIOS Klinikum Berlin-Buch, †Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany

ABSTRACT
The prorenin/renin receptor is a recently discovered component of the renin-angiotensin system. The effects of aliskiren, a direct inhibitor of human renin, were compared with the handle region decoy peptide (HRP), which blocks the prorenin/renin receptor, in double-transgenic rats overexpressing the human renin and angiotensinogen genes. After 7 wk, all aliskiren-treated rats were alive, whereas mortality was 40% in vehicle-treated and 58% in HRP-treated rats. Aliskiren but not the HRP reduced BP and normalized albuminuria, cystatin C, and neutrophil gelatinase-associated lipocalin, a marker of renal tubular damage, to the levels of nontransgenic controls. In vitro, human renin and prorenin induced extracellular signal–regulated kinase 1/2 phosphorylation, independent of angiotensin II (AngII), in vascular smooth muscle cells. Preincubation with the HRP or aliskiren did not prevent renin- and prorenin-induced extracellular signal–regulated kinase 1/2 phosphorylation, whereas the MAP kinase kinase (MEK1/2) inhibitor PD98059 prevented both. In conclusion, renin inhibition but not treatment with the HRP protects against AngII-induced renal damage in double-transgenic rats. In addition, the in vitro data do not support the use of the HRP to block AngII-independent prorenin- or renin-mediated effects.

Deterioration of Kidney Function by the (Pro)renin Receptor Blocker Handle Region
Peptide in Aliskiren-treated Diabetic Transgenic (mRen2)27 Rats

Luuk te Riet, Mieke van den Heuvel, Carine J. Peutz-Kootstra,
Joep H.M. van Esch, Richard van Veghel, Ingrid M. Garrelds, Usha Musterd-Bhaggoe,
Angelique M. Bouhuizen, Frank P.J. Leijten, A.H. Jan Danser & Wendy W. Batenburg
Division of Pharmacology and Vascular Medicine, Department of Internal Medicine, Erasmus MC, The Netherlands and *Department of Pathology, Maastricht University Medical Center, Maastricht, The Netherlands

Short title: (Pro)renin receptor, heart and kidney
Word count: 6343

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Fax: +31-10-7044733
E-mail: w.batenburg@erasmusmc.nl
CD Expression of Renin

Stimuli:
- Low salt
- AngII
- Diabetes

Rohrwasser A et al. *Hypertension* 1999
Quantification of intensity of distal nephron renin immunoreactivity in rat kidney cortex and medulla of Ang II–infused and control rats

Prieto-Carrasquero M C et al. *Hypertension* 2004
Distinct Regulation of Renal Cortical and Medullary Renin Activity in AngII Hypertension in Rats

A

Plasma renin activity (AngI ng/hr/µg)

CTR

AngII

P<0.05

B

Urine renin activity (AngI ng/24hr)

CTR

AngII

P<0.05

C

Renal cortical renin activity (AngI ng/hr/µg)

CTR

AngII

P<0.05

D

Renal inner medullary renin activity (AngI ng/hr/µg)

CTR

AngII

P<0.05
Does PRR regulate intrarenal RAS in AngII-induced hypertension?
Role of Intrarenal RAS in AngII-Induced Hypertension

- AngII content in renal tissues is much higher than that in the plasma and it is 4-5 times higher in the medulla than in the cortex (Navar 1997)
- When endogenous AngII production was reduced by ACE inhibition, AngII–infused mice became normotensive (Gonzalez-Villalobos 2010)
- The genetic absence of kidney ACE substantially blunts the hypertension induced by AngII infusion (Gonzalez-Villalobos 2013)
- CD deletion of renin attenuates AngII-induced hypertension (Ramkumar 2014)
Tissue Distribution of COX Isoform Proteins in Rats

Immunoblotting

- Brain
- Spleen
- Renal Cortex
- Renal Inner Medulla
- Heart
- Liver
- Stomach
- Small Intestine
- Lung
- Testis

COX-2

- 72 KD

COX-1

- 70 KD

Yang AJP-Renal 1998
Immunostaining of COX-2 in renal medulla of salt treated Sprague-Dawley Rats

COX-2

COX-1

Ye et al. AJP Renal 2005
Time Course of AngII Stimulation of PRR Expression in Primary Rat IMCD Cells

<table>
<thead>
<tr>
<th>Time</th>
<th>PRR</th>
<th>β-Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>43 kDa</td>
<td>42 kDa</td>
</tr>
<tr>
<td>6 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Basis of Diversified Actions of PGE2

15-PGDH, NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH)

OAT-PG, Prostaglandin-specific organic transporter

PGE2

EP1

EP3

EP2

EP4

Gq

Gi

Gs

Gs

[Ca^{++}]_i

cAMP

cAMP

cAMP

Vasoconstriction

Vasorelaxation
Plasma Renin Activity Induced by Bumetanide Infusion

- Basal vs. WT Bum: p<0.0001
- Bum vs. WT Bum: p=0.55

WT COX2-/- COX1 -/-
Summary of Results with 3 EP Antagonists

- Vehicle Control
- EP1 A
- EP3 A
- EP4 A

*P* < 0.01

*P* < 0.05

*P* > 0.05
ELISA Detection of Prorenin/Renin

### IM
- Renal inner medullary total Prorenin/Renin (ng/μg)
  - CTR: 70 ± 10
  - AngII: 150 ± 20
  - AngII + ONO: 60 ± 10

### Plasma
- Plasma total Prorenin/Renin (ng/μg)
  - CTR: 20 ± 5
  - AngII: 25 ± 5
  - AngII + ONO: 20 ± 5

### Urine
- Urine total Prorenin/Renin (μg/24hr)
  - CTR: 100 ± 20
  - AngII: 200 ± 50
  - AngII + ONO: 100 ± 20

### Coretx
- Renal cortical total Prorenin/Renin (ng/μg)
  - CTR: 30 ± 5
  - AngII: 15 ± 5
  - AngII + ONO: 20 ± 5

Significance:
- P<0.05 for all comparisons
High Salt Stimulates COX-2 Expression in Renal Inner Medulla of Sprague-Dawley Rats

Immunoblotting

<table>
<thead>
<tr>
<th></th>
<th>Low Salt</th>
<th>Normal Salt</th>
<th>High Salt</th>
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<tbody>
<tr>
<td>COX-2</td>
<td></td>
<td>72 kd</td>
<td></td>
</tr>
<tr>
<td>COX-1</td>
<td></td>
<td>70 kd</td>
<td></td>
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</tbody>
</table>

Yang et al. AJP 1998
Effects of Intramedullary Infusion of NS-398 on MAP in High Salt Treated Rats

Ye et al. AJP Renal 2005
Renal Renin mRNA Expression in Rats after 2 Weeks of AngII Infusion and/or ONO-AE3-208 Treatment
Nguyen 2002

Angiotensinogen

prorenin → renin → Ang I

ACE

PRR → Ang II

AT1R

Vascular dysfunction
Neuroglial dysfunction
Inflammation
Oxidative damage

ACE2

Ang (1-7)

Mas

Anti-angiogenic
Neuroprotective
Anti-inflammatory
Anti-oxidative
Systolic Blood Pressure

Wang F et al. *AJP-Renal* 2014
Active and Total Renin in the Renal Inner Medulla and Urine

Wang et al. *Hypertension* 2014
(Pro)Renin Receptor (ATP6AP2) Signaling via Wnt/β-catenin in Low Vertebrates
Non-Classical RAS and New Drug Development

- AGT
- AngI
- AngII
- AngIII
- AngIV
- Endopeptidase
- Ang1-7 analog (preclinical)
- Ang1-7
- rhACE2 (phase I)
- ACE2
- ACE
- ACE
- AngII
- AngIII
- AngIV
- AT1
- AT2
- AT4/IRAP
- Mas
- AVE0991, CGEN-88565 (preclinical)
- Mas
- C21 (preclinical)
- HRP PRO20 (preclinical)
- RB150 (phase I)
- PC18 (preclinical)
- PRR
- ROS/MAPK
Renin activity

Plasma renin activity (Ang I ng/ml/h)

- **PRR Floxed**
  - CTR
  - AngII

- **PRR KO**
  - CTR
  - AngII

Urinary renin activity (Ang I ng/24h)

- **PRR Floxed**
  - CTR
  - AngII

- **PRR KO**
  - CTR
  - AngII
Contribution of PRR to AVP-induced AQP2 expression in primary rat IMCD cells

CTR AVP AVP+PRO20

AQP2

β-Actin

CTR AVP AVP+ Anti-PRR

AQP2

β-Actin

CTR AVP AVP+ siRNA

AQP2

β-Actin
Institute of Hypertension, Sun Yat-sen University
School of Medicine, Guangzhou, China
Institute of Hypertension, Sun Yat-sen University
School of Medicine, Guangzhou, China
The Native sPRR Upregulate ENaC Expression

CTR sPRR (from mice) sPRR (from human)

α-ENaC

CTR  sPRR (mouse)  sPRR (human)

β-Actin

95 kDa

43 kDa

ENaC densitometry/β-actin
OMP and ICG Blocked sPRR-His Induced β-Catenin Signaling Activation in mpkCCD cells

Luminescence assay for LCF/TCF activity

**OMP**

CTR

sPRR-His

sPRR-His + OMP

---

**ICG**

CTR

sPRR-His

sPRR-His + ICG

OMP: Frizzled-8 inhibitor

ICG: β-catenin inhibitor
OMP and ICG Blocked sPRR-induced α –ENaC mRNA Expression in mpkCCD Cells
IV sPRR-His Rescued the Blunted Hypertensive Response in CD PRR KO mice

MAP (mmHg)

Day of treatment

sPRR-His infusion

AngII infusion

* * *

# # #
Published Articles on Renal PRR
 Canonical Wnt Signaling

OFF STATE

cadherin
LRP5/6

DKK
Frizzled

Tankyrase — VAX-939

β-catenin
α-catenin

CK1
GSK
AXIN

β-catenin

β-TrCP

Proteasome

β-catenin degradation

groucho
TCF/LEF

Sino Biological provides scientists around the world
β-Catenin Regulation of AVP Signaling: AQP2 Trafficking or Transcription?
Functions of Renal RRR in Regulation of Na\textsuperscript{+} and Water and Blood Pressure Homeostasis

Intrarenal RAS activity

BP regulation

AngII signaling

ENaC regulation

PRR

Water transport

AVP signaling

K\textsuperscript{+} transport

sPRR signaling

Yang lab
2. Wang F et al. AJP-Renal 2014
4. Lu X et al. AJP-Renal 2015
5. Peng K et al. AJP-Renal 2016
7. Lu X et al. PNAS 2016
8. Xu C et al. AJP-Renal 2016

Kohan/Ramkumar lab
9. Ramkumar N et al. AJP-Renal 2015
10. Ramkumar N et al. AJP-Renal 2015

Siragy lab

Prieto lab

Simons lab
Diabetes Insipidus in CD PRR KO Mice

Wang F et al. JASN 2016
Downregulation of Renal Medullary AQP2 Expression in CD PRR KO Mice

Wang F et al. JASN 2016