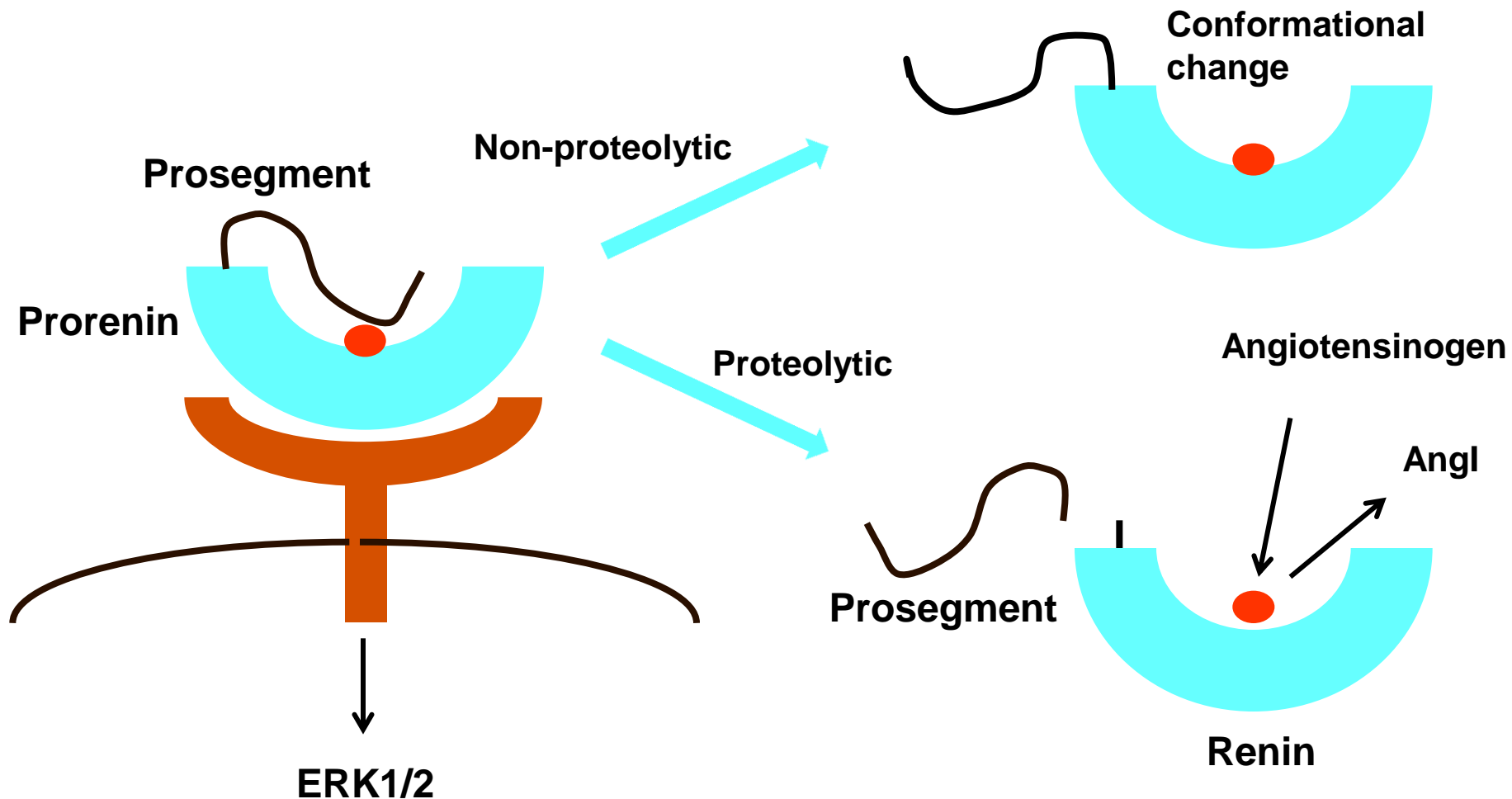


Does (Pro)Renin Receptor Deserve Its Name?

Tianxin Yang, MD/PhD

**Department of Internal Medicine, University of Utah;
Institute of Hypertension, Sun Yat-sen University**

In Vitro Evidence for Renin Regulatory Property of PRR



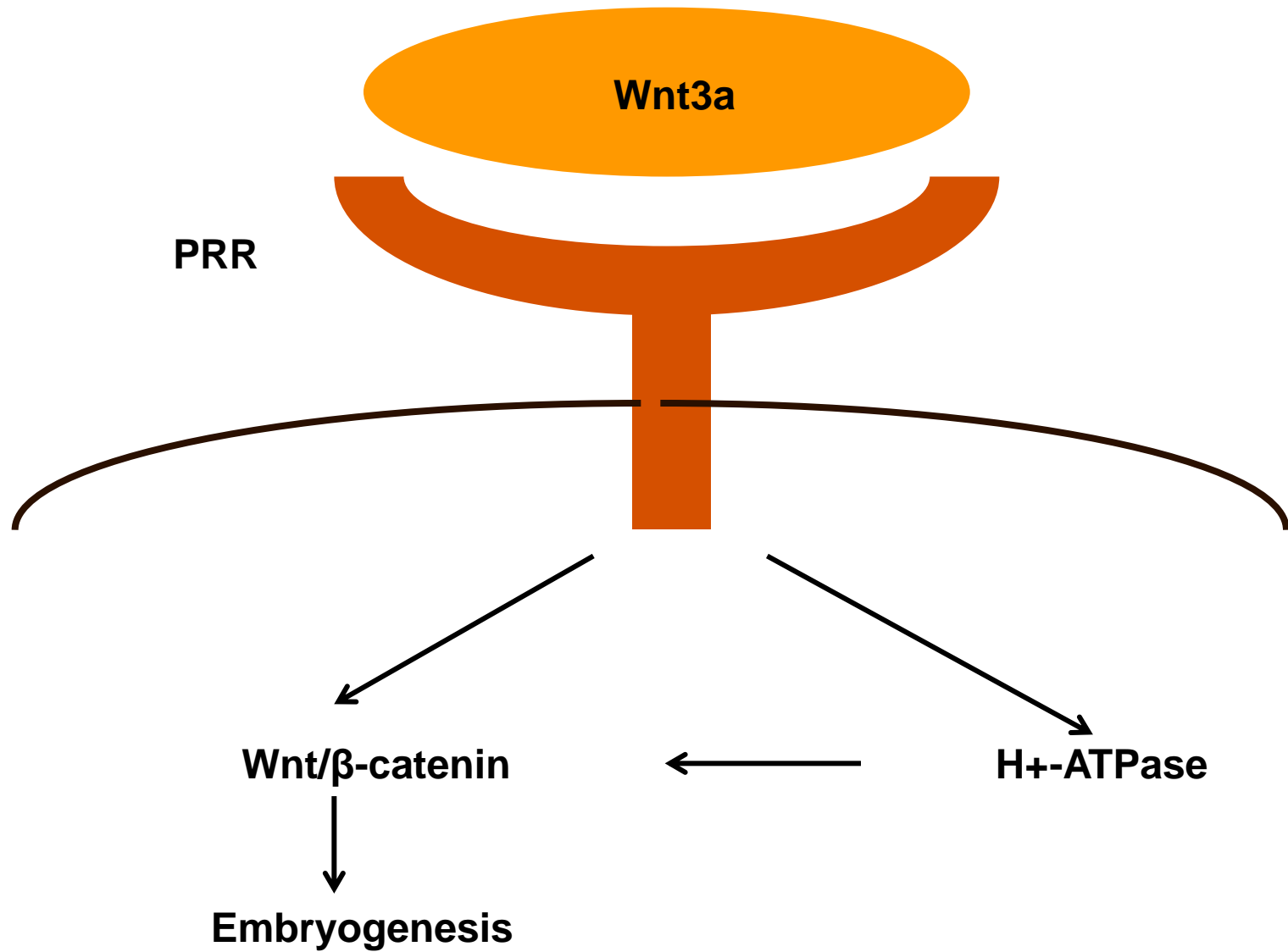
Lethal Phenotype in Various PRR KO Mice

- Conventional PRR KO
- Cardiomyocyte PRR KO
- Podocyte PRR KO
- CD PRR KO (Hoxb7 Cre)

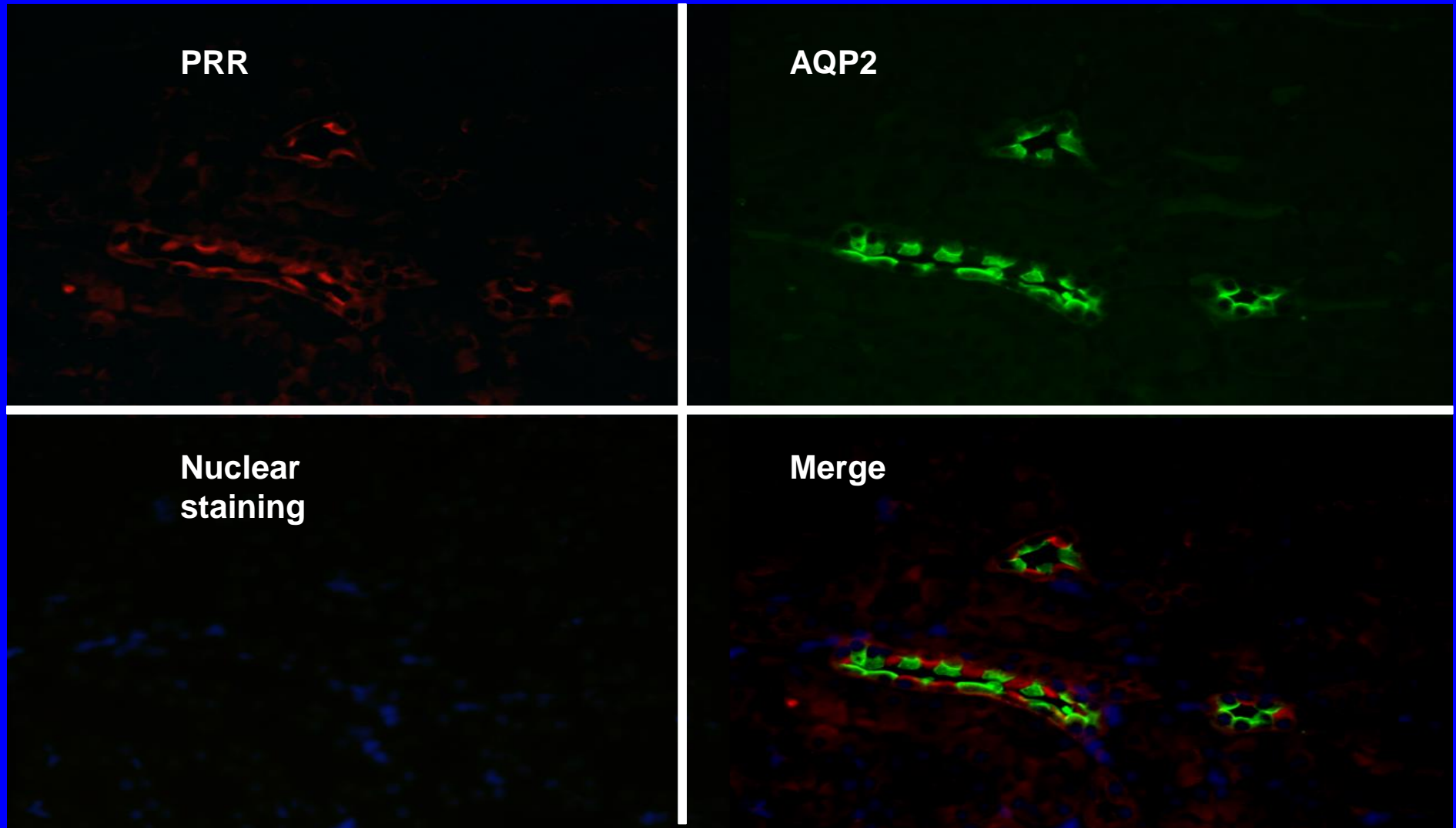
Evidence Arguing against PRR as a Renin Regulator

- Overexpression of human PRR in rats resulted in proteinuria and nephropathy but did not elevate BP or renal AngII levels (Kaneshiro Y 2007)
- Challenges: Lack of viable KO; controversial handle region peptide (HRP).

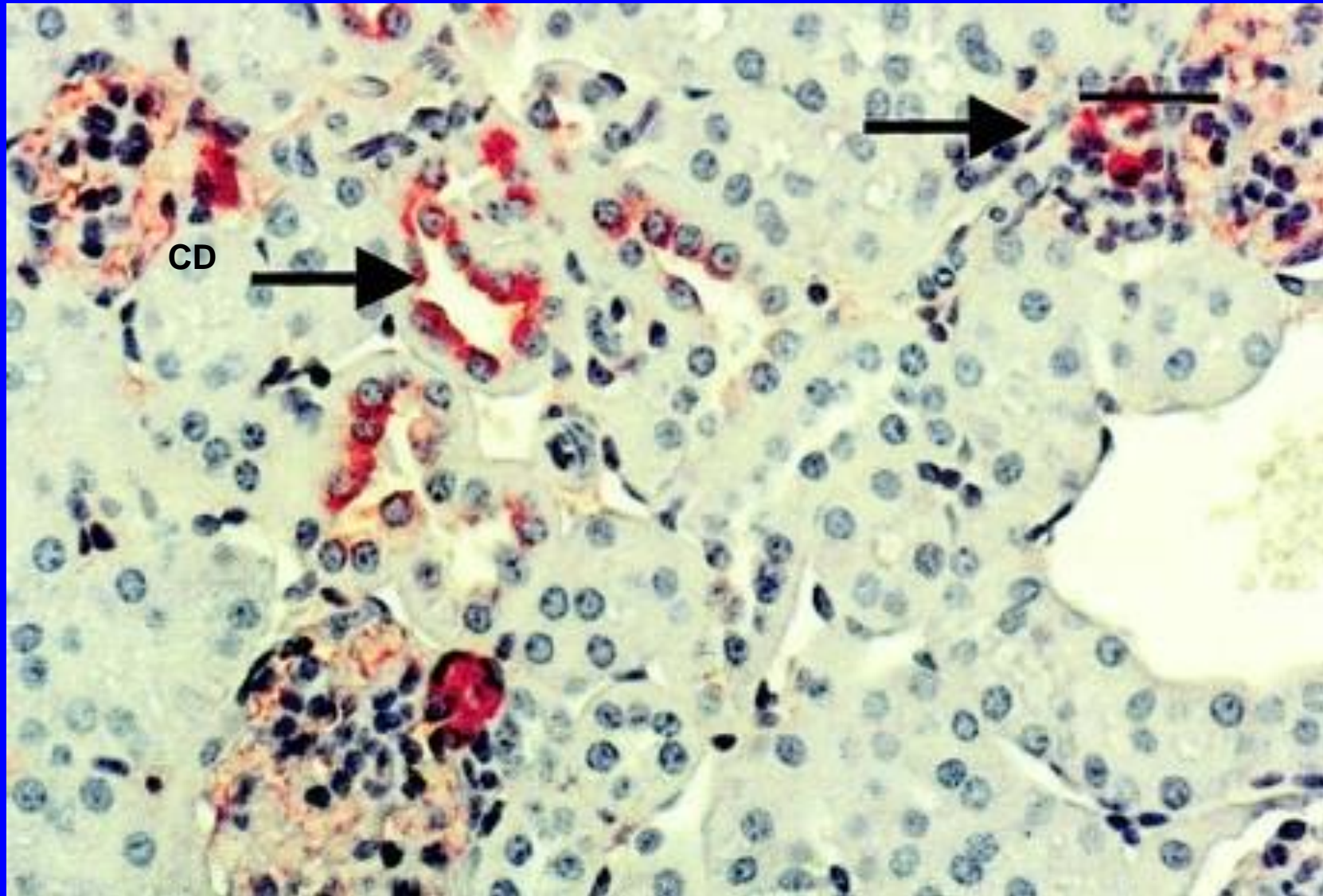
(Pro)Renin Receptor (ATP6AP2) Signaling via Wnt/ β -catenin in Low Vertebrates



Localization of PRR in the Collecting Duct Intercalated Cells



CD Expression of Renin



COX-2/PGE2 Pathway Mediates AngII-Induced Renin Response and PRR Expression in CD Cells

COX-2 mediates angiotensin II-induced (pro)renin receptor expression in the rat renal medulla

Fei Wang,^{1,2} Xiaohan Lu,^{1,2} Kevin Peng,^{1,3} Li Zhou,¹ Chunling Li,¹ Weidong Wang,¹ Xueying Yu,² Donald E. Kohan,³ Shu-Feng Zhou,¹ and Tianxin Yang^{1,3}

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Wang F, Lu X, Peng K, Zhou L, Li C, Wang W, Yu X, Kohan DE, Zhu SF, Yang T. COX-2 mediates angiotensin II-induced proteinase receptor expression in the rat renal medulla. *Am J Physiol Renal Physiol* 307: F25–F32, 2014. doi:10.1152/ajprenal.00484.2013. First published April 16, 2014.

Angiotensin II (Ang II) is predominantly expressed in the distal nephron where it is activated by angiotensin II (Ang II), resulting in increased renin activity in the renal medulla thereby amplifying the de novo generation and action of local Ang II. The goal of the present study was to test the role of cyclooxygenase-2 (COX-2) in mediating Ang II-induced PRR expression in the renal medulla *in vitro* and *in vivo*. Exposure of primary rat inner medullary collecting duct cells to Ang II induced sequential increases in COX-2 and PRR protein expression. When the cells were pretreated with a COX-2 inhibitor NS-398, Ang II-induced upregulation of PRR protein expression was almost completely abolished, in parallel with the changes in medullary active renin content. The inhibitory effect of NS-398 on the PRR expression was reversed by adding exogenous PGE₂. A 14-day Ang II infusion elevated renal medullary PRR expression and active and total renin content in parallel with increased urinary renin, all of which was remarkably suppressed by the COX-2 inhibitor celecoxib. In contrast, plasma and renal cortical active and total renin content were suppressed by Ang II treatment, an effect that was unaffected by COX-2 inhibition. Systolic blood pressure was elevated with Ang II infusion, which was attenuated by the COX-2 inhibitor. Overall, the results obtained from *in vitro* and *in vivo* studies established a crucial role of COX-2 in mediating upregulation of renal medullary PRR expression and renin content during Ang II hypertension.

(pro)renin receptor, renin activity, cyclooxygenase-2, inner medullary collecting duct, prostaglandin E₂

THE RENIN-ANGIOTENSIN SYSTEM (RAS) plays a pivotal role in the regulation of blood pressure, cardiovascular function, renal hemodynamics, and tubular sodium reabsorption (8). Compared with the well-recognized role of systemic RAS in regulation of blood pressure and in the pathogenesis of hypertension, in recent years, there has been increasing appreciation of the potential role of local RAS found in a variety of tissues including the brain, heart, adrenal glands, vasculature, and the kidney (7, 18). The evidence of intrarenal RAS is highlighted by the discovery of renin expression in the connecting tubules and cortical and medullary collecting ducts (CDs) (12, 24) and angiotensinogen expression in the proximal tubule (11), the

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F25

Renin-Angiotensin System

Prostaglandin E-Prostanoid₂ Receptor Mediates Angiotensin II-Induced (Pro)Renin Receptor Expression in the Rat Renal Medulla

Fei Wang, Xiaohan Lu, Kevin Peng, Yaomin Du, Shu-Feng Zhou, Aihua Zhang, Tianxin Yang

Abstract—Angiotensin II (Ang II) stimulates (pro)renin receptor (PRR) expression in the renal collecting duct, triggering the local renin response in the distal nephron. Our recent study provided evidence for involvement of cyclooxygenase-2-prostaglandin E₂ pathway in Ang II-dependent stimulation of PRR expression in the collecting duct. Here, we tested the role of E-prostanoid (EP) subtypes acting downstream of cyclooxygenase-2 in this phenomenon. In primary rat inner medullary collecting duct cells, Ang II treatment for 12 hours induced a 1.8-fold increase in the full-length PRR protein expression. To assess the contribution of EP receptor, the cell was pretreated with specific EP receptor antagonists: SC-51382 (for EP₁), L-798106 (for EP₂), L-161982 (for EP₃), and ONO-AE5-208 (ONO, a structurally distinct EP₂ antagonist). The upregulation of PRR expression by Ang II was consistently abolished by L-161982 and ONO and partially suppressed by SC-51382 but was unaffected by L-798106. The PRR expression was also significantly elevated by the EP₂ agonist CAY10598 in the absence of Ang II. Sprague-Dawley rats were subsequently infused for 1 or 2 weeks with vehicle, Ang II alone, or in combination with ONO. Ang II infusion induced parallel increases in renal medullary PRR protein and urinary renin activity and total renin content, all of which were blunted by ONO. Both tail cuff plethysmography and telemetry demonstrated attenuation of Ang II hypertension by ONO. Overall, these results have established a crucial role of EP₂ receptor in mediating the upregulation of renal medullary PRR expression and renin activity during Ang II hypertension. (*Hypertension*. 2014;64:369–377.)

Key Words: diuresis

In recent years, there has been rising interest about the local renin-angiotensin system (RAS) in a variety of tissues including the kidney.¹² Within the kidney, angiotensinogen is expressed in the proximal tubule and renin in the connecting tubules¹¹ and cortical and medullary collecting ducts (CDs),²⁴ forming the anatomic basis of intrarenal RAS. In response to angiotensin II (Ang II), the intrarenal RAS is activated as reflected by increased renin mRNA and protein expression in the CD,²⁴ whereas the systemic RAS is suppressed, highlighting the difference in the 2 RAS system. Several lines of evidence demonstrate a critical role of intrarenal RAS in Ang II-induced hypertension. Experiments in rats infused with Val¹-Ang II, an isoform of Ang II that can be separated from endogenous Ang II (10³-Ang II) by high-performance liquid chromatography, demonstrated that the chronic Val¹-Ang II (exogenous Ang II) infusion induces renal 10³-Ang II (endogenous Ang II) synthesis.¹³ In another study, when endogenous Ang II production was reduced by angiotensin-converting enzyme inhibition, Ang II-induced mice became normotensive.¹⁴ The genetic absence of kidney angiotensin-converting

enzyme substantially blunts the hypertension induced by Ang II infusion.¹⁵ In experiments involving kidney cross-transplantation between global angiotensin II receptor-1 knockout mice and wild-type controls, Ang II shows to cause hypertension through stimulation of angiotensin II receptor-1 receptors in the kidney.¹⁶ Last, overexpression of renin in the CD causes spontaneous hypertension.¹⁷ However, evidence also exists to suggest that some components of the RAS may be of extrarenal origin. For example, renal angiotensinogen and Ang II are shown to originate from liver.¹⁸

(Pro)renin receptor (PRR) is a newly discovered component of the RAS, being capable of binding renin and prorenin with almost equal affinity to increase their catalytic activity.¹⁹ PRR is considered to play an important role in regulation of tissue renin activity, thereby controlling the activity of local RAS. Within the kidney, PRR expression is predominantly expressed in the intercalated cells of the CD.¹³ Chronic infusion of Ang II in rats increased renal PRR transcript levels and augmented the PRR activity in renal medullary tissues, which may contribute to increased renin activity in the CD during Ang II hypertension (7). The activation of renal medullary PRR is considered as a key component of the local renin response that may participate in regulation of blood pressure and fluid metabolism during Ang II hypertension. However, the mechanism of how Ang II stimulates PRR expression in the CD is unknown.

Prostanoids including PGE₂, PGE₁, PGI₂, and thromboxane A₂ are derivatives of arachidonic acid through the activity of constitutive isoform COX-1 or the inducible isoform COX-2 (29). Prostanoids participate in blood pressure control by acting on the kidneys, blood vessels, endocrine organs, and brain. Despite the initial characterization as inducible cyclooxygenase, COX-2 has been recognized as an important regulator of renin secretion, renal function, and blood pressure (4, 9). In particular, at basal condition, COX-2 is abundantly expressed in the renal medulla where its expression is elevated by salt loading (5, 30). Renal medullary COX-2 plays an important role in regulation of renal medullary blood flow or tubular sodium transport (22). In the hypertension model induced by chronic Ang II infusion, COX-2 deficiency

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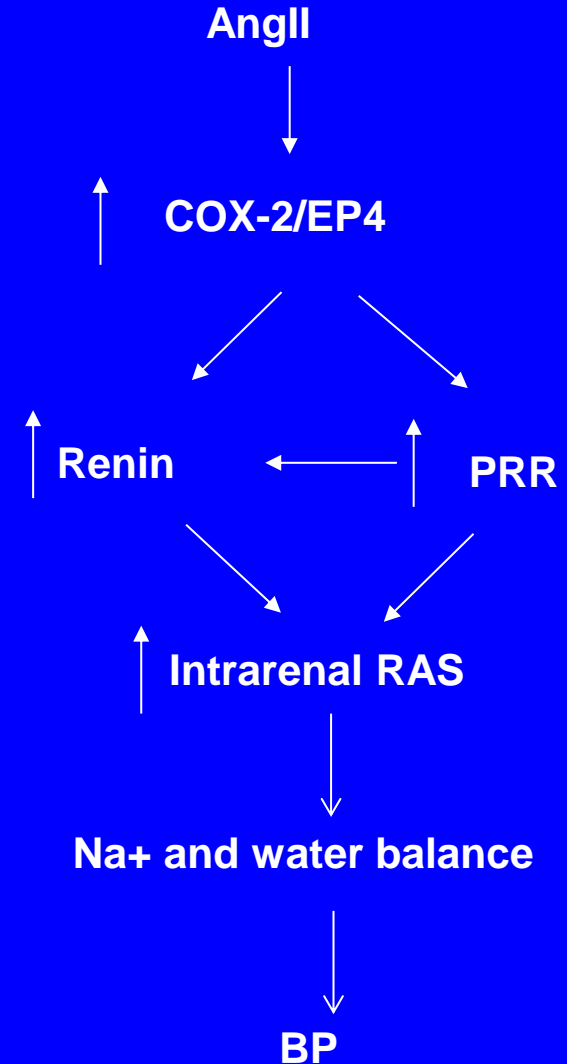
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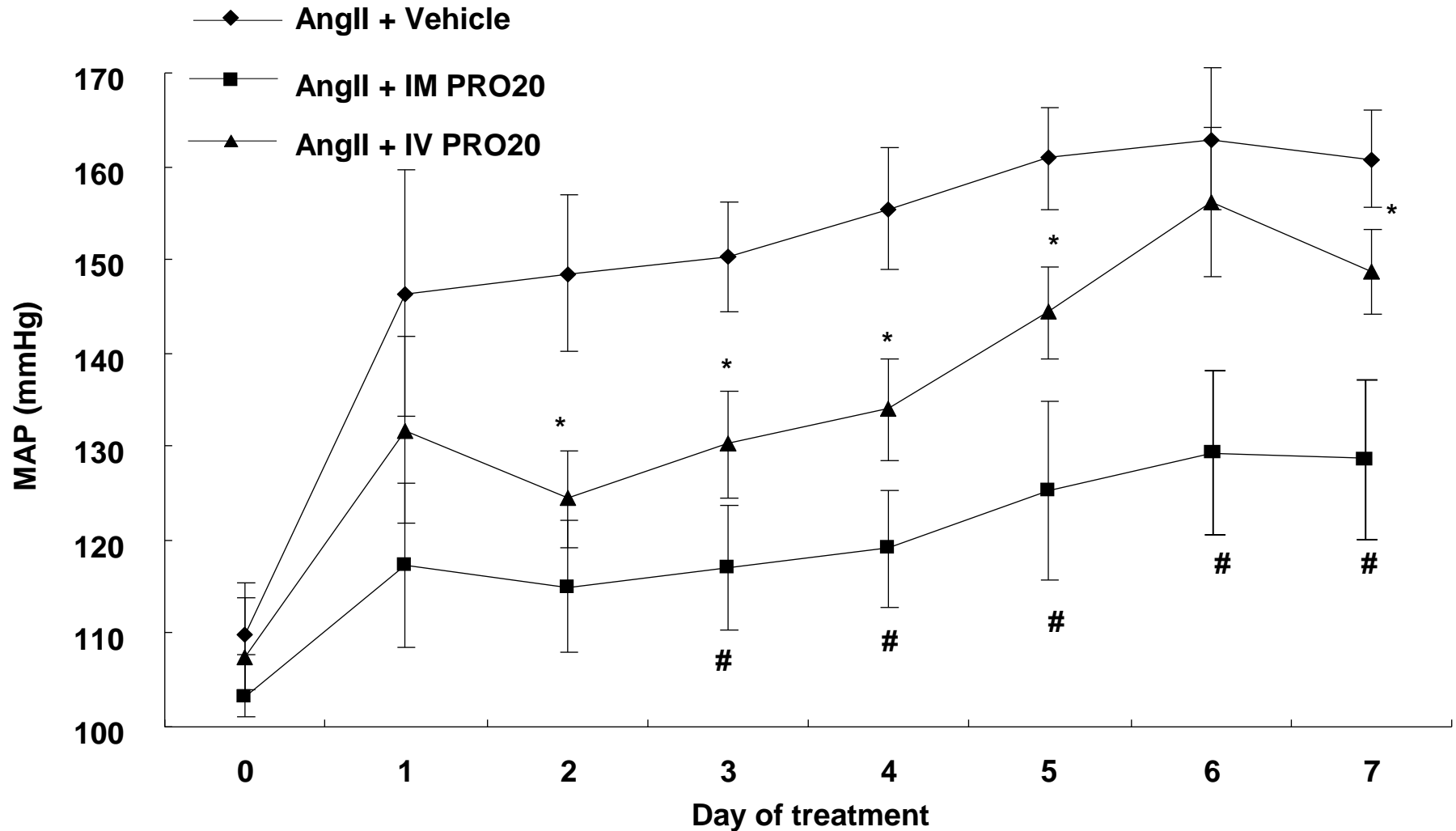
Hypertension is available at <http://hyper.ahajournals.org>

DOI: 10.1161/HYPERTENSIONAHA.114.08654

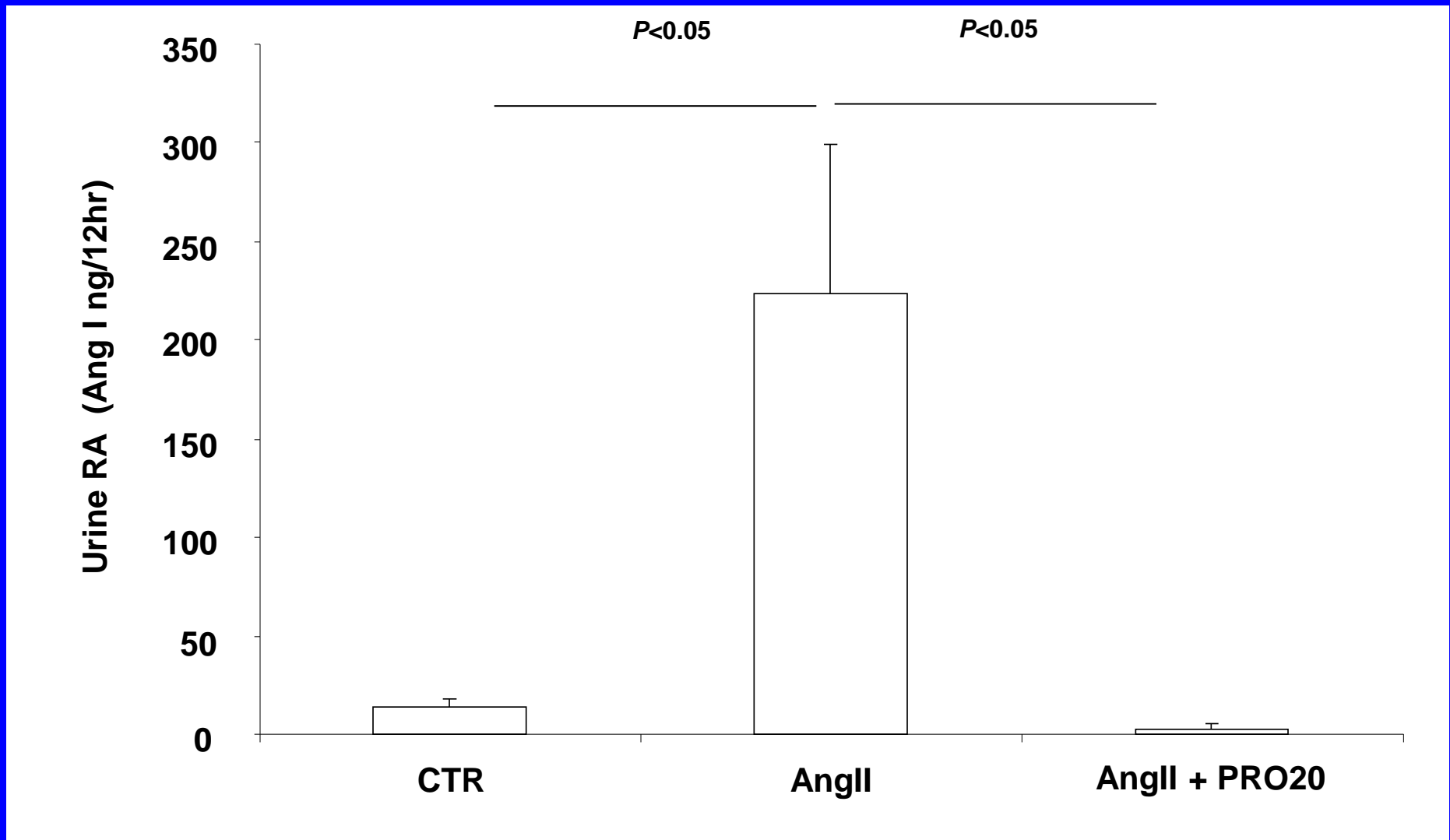
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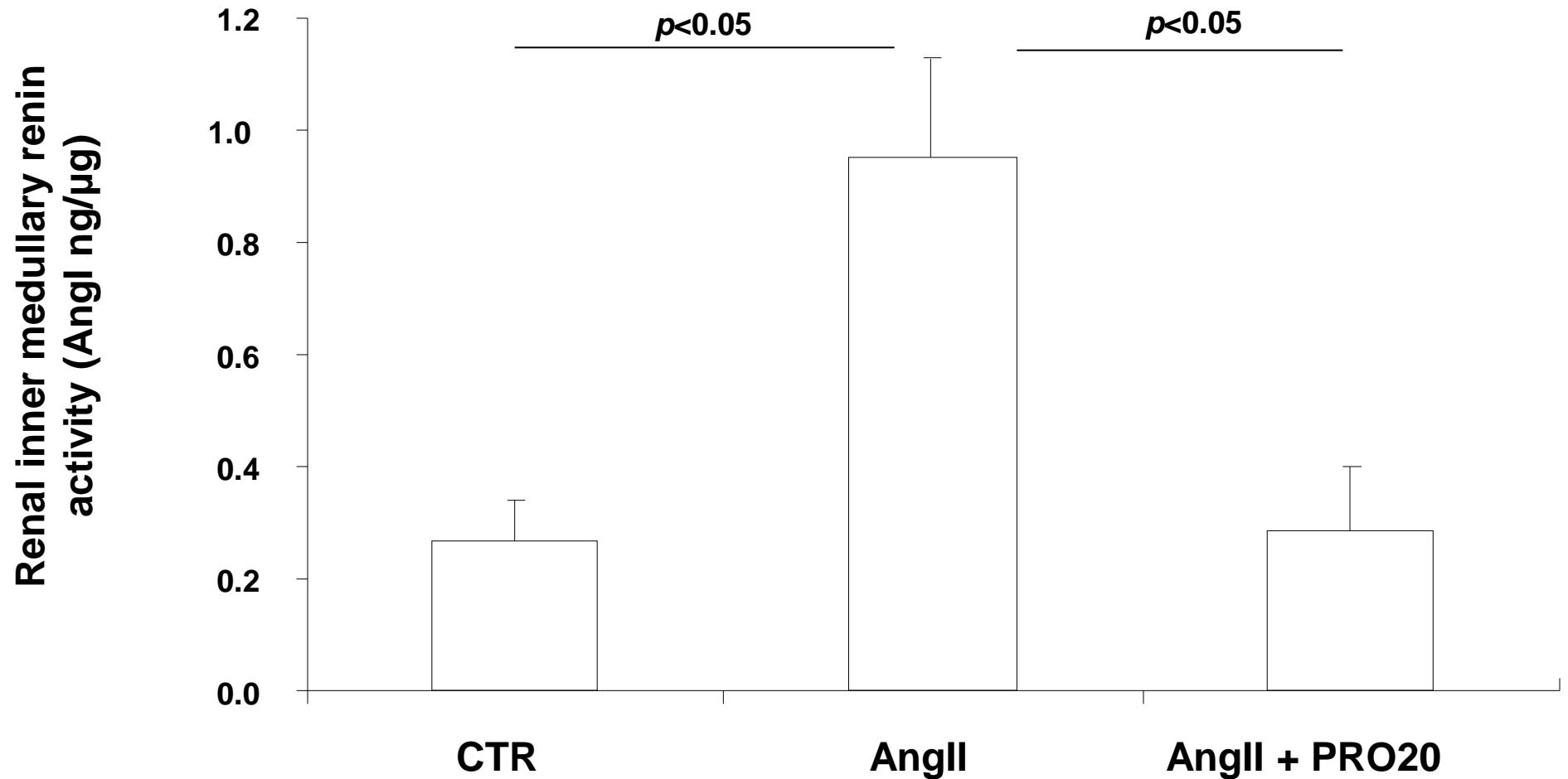
MAP in Rats Receiving Intramedullary or Intravenous Infusion of PRO20



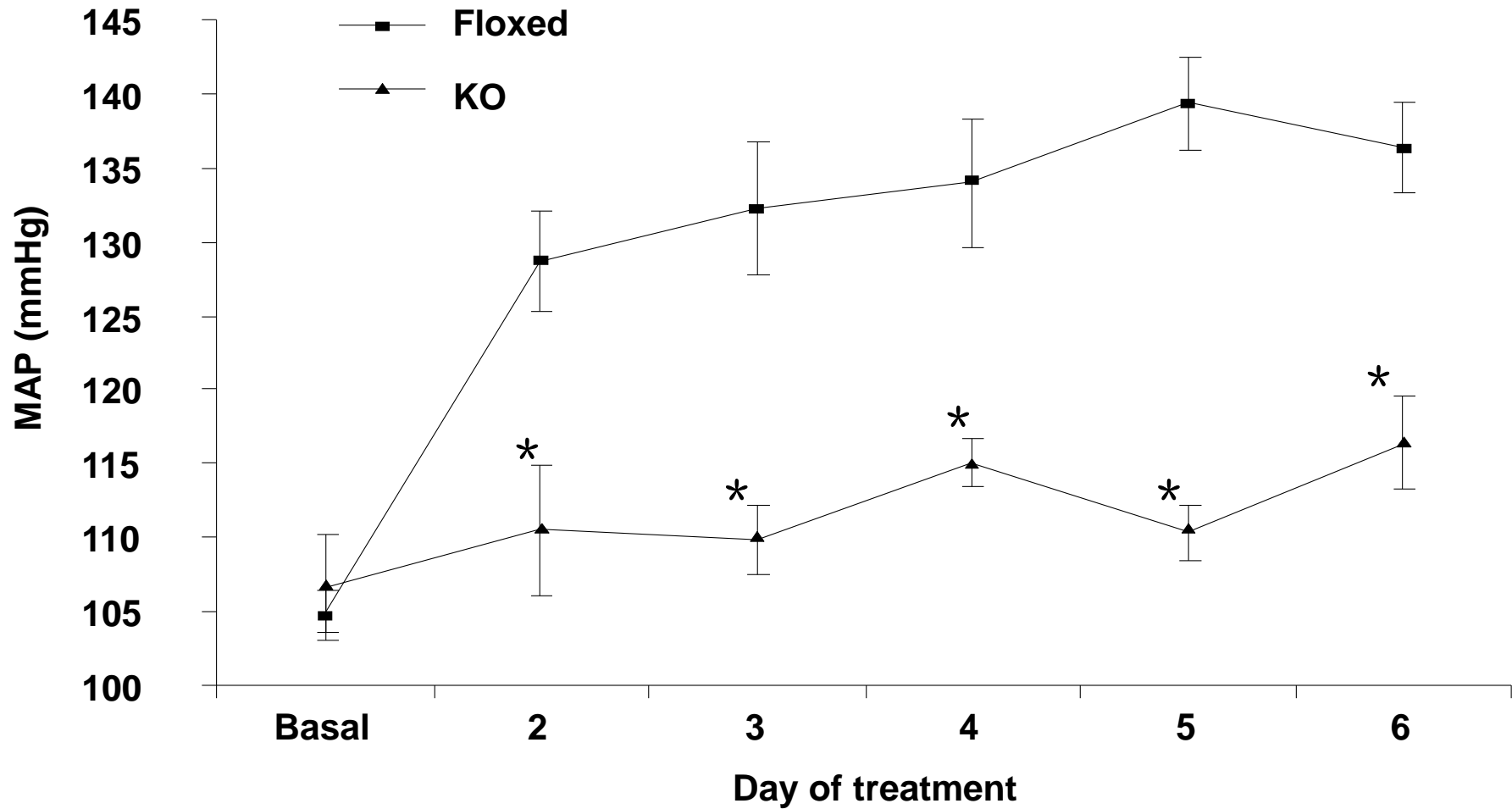
Effect of Intramedullary Infusion of A PRR Blocker on AngII-Induced Urinary Renin Activity



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



MAP in CD PRR KO mice after AngII Infusion



Osmoreceptors detect increased osmotic pressure

Baroreceptors (aortic arch, carotid sinus) detect decreased blood pressure

Hypothalamic neuron

SON
PVN

Posterior pituitary

ADH

V2R

V1a

Vasopressin

Increased reabsorption of water

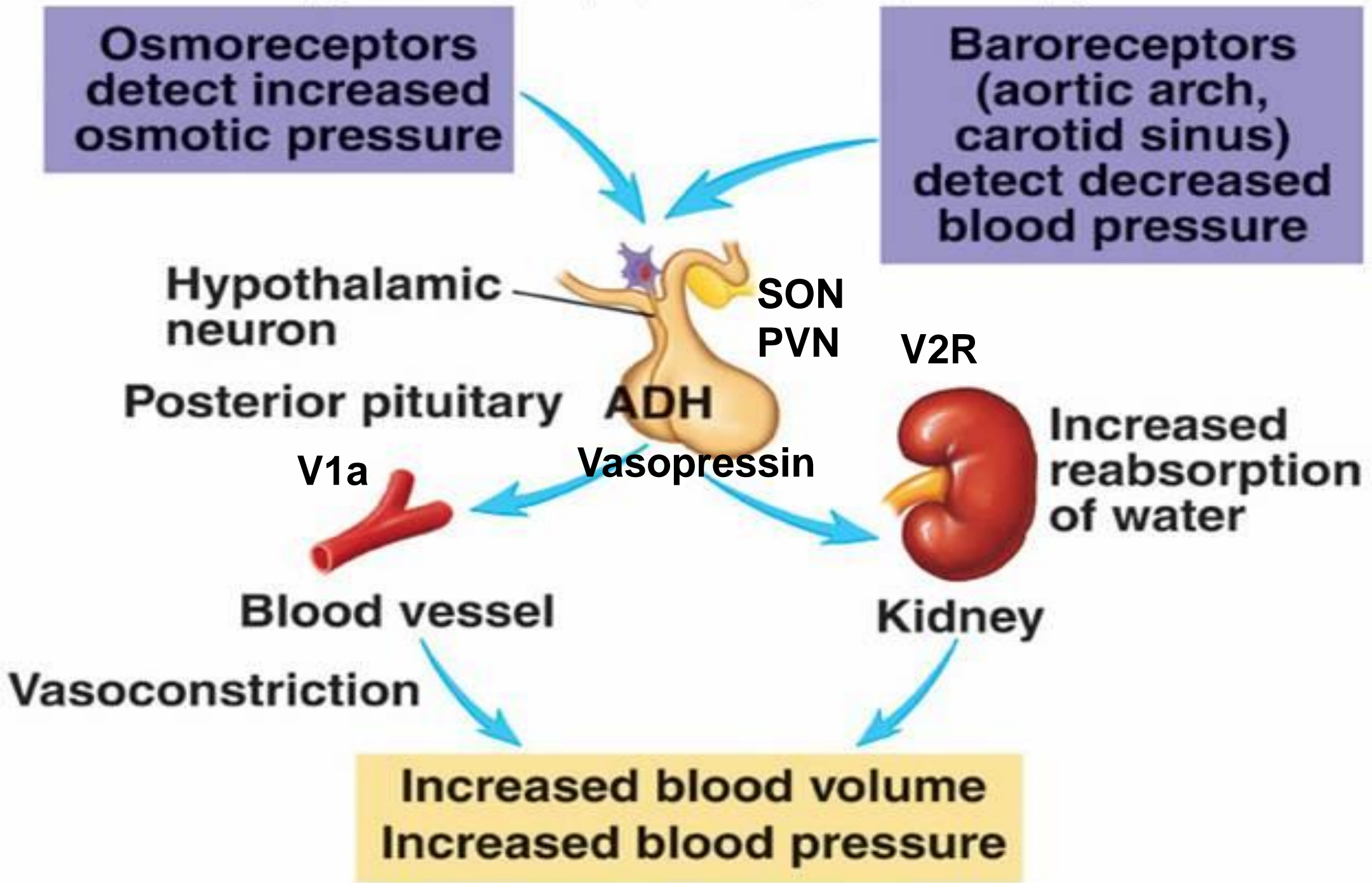


Blood vessel

Kidney

Vasoconstriction

Increased blood volume
Increased blood pressure

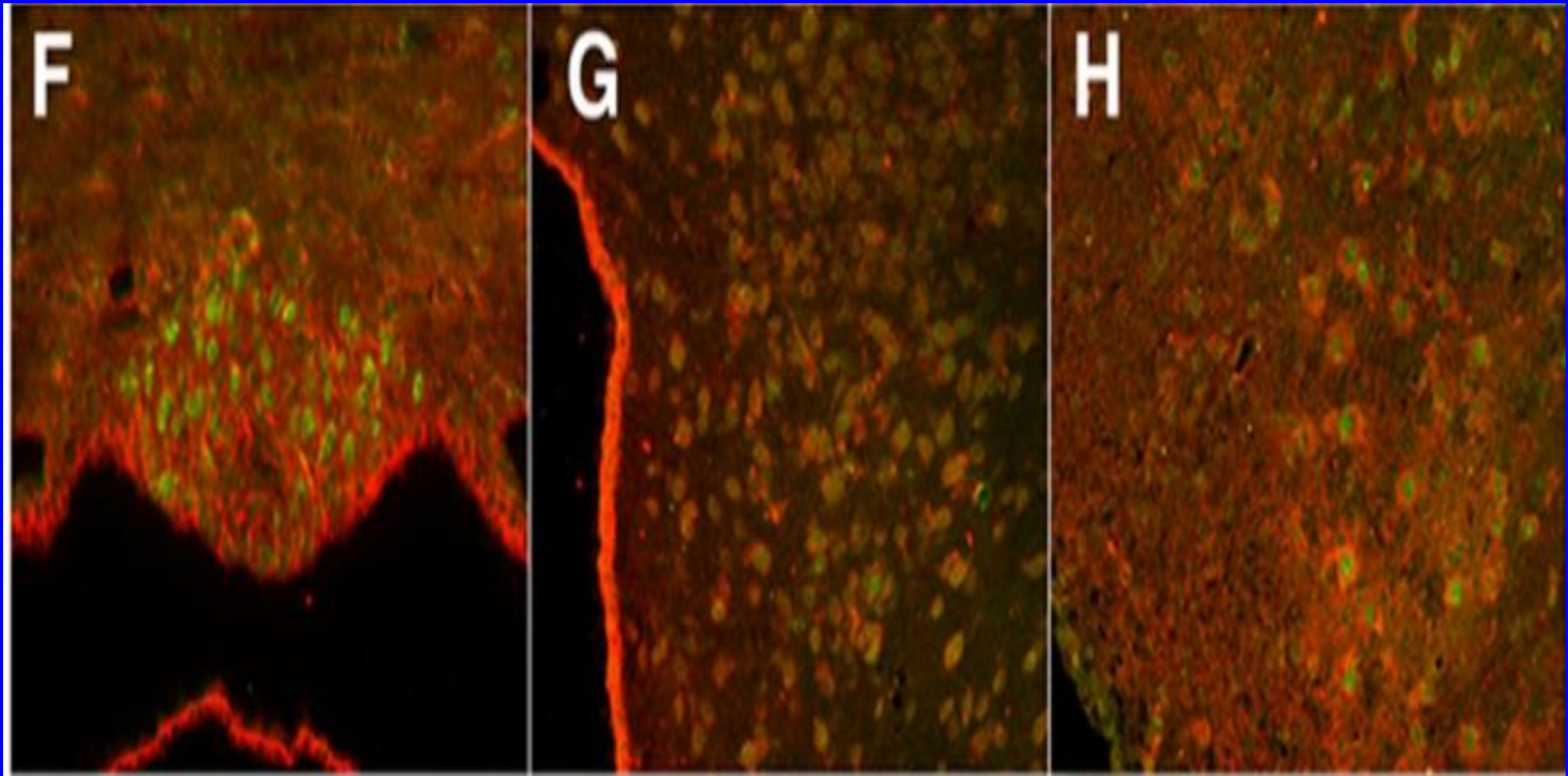


Immunostaining of PRR in Rat Brain

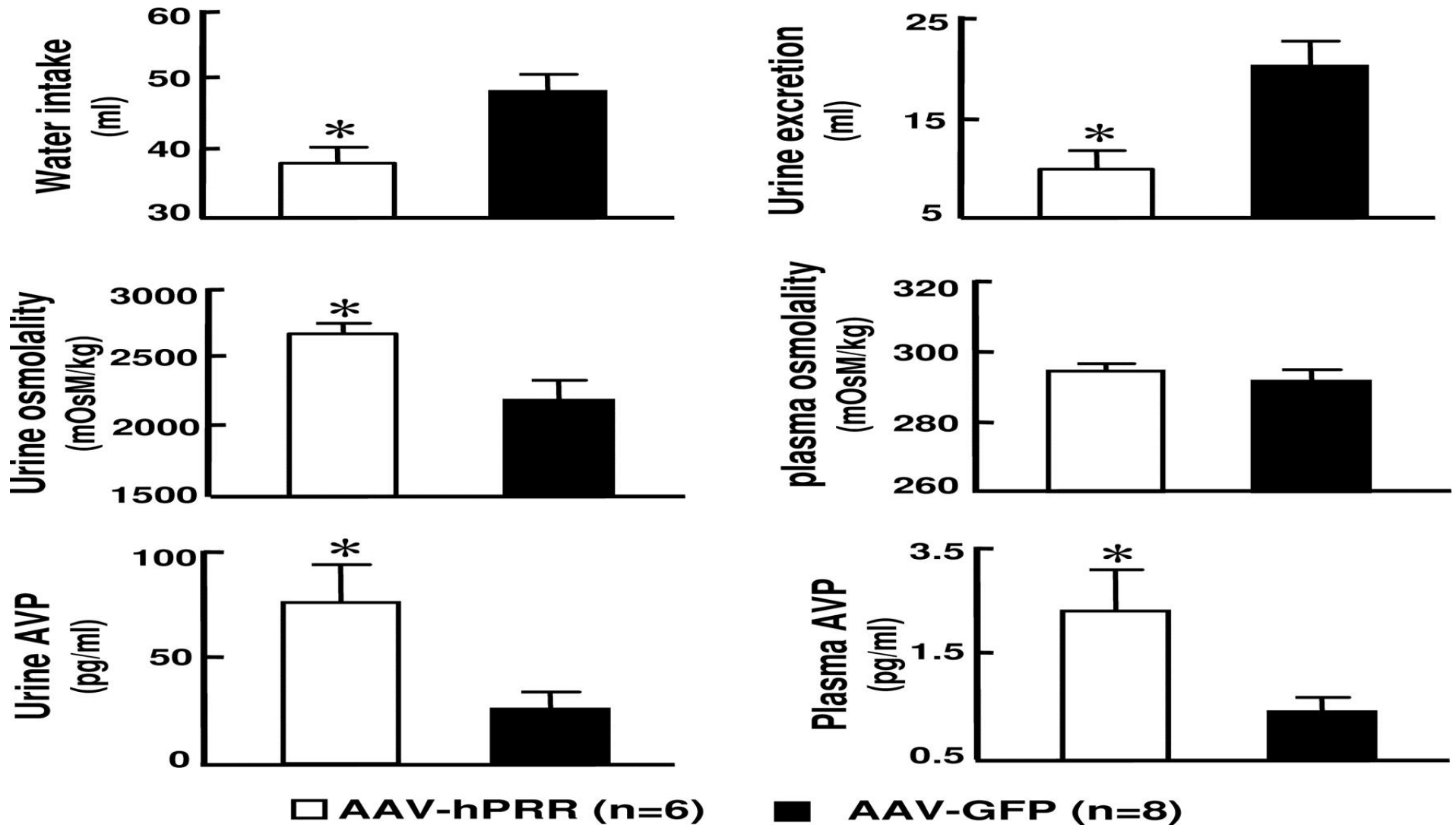
SFO

PVN

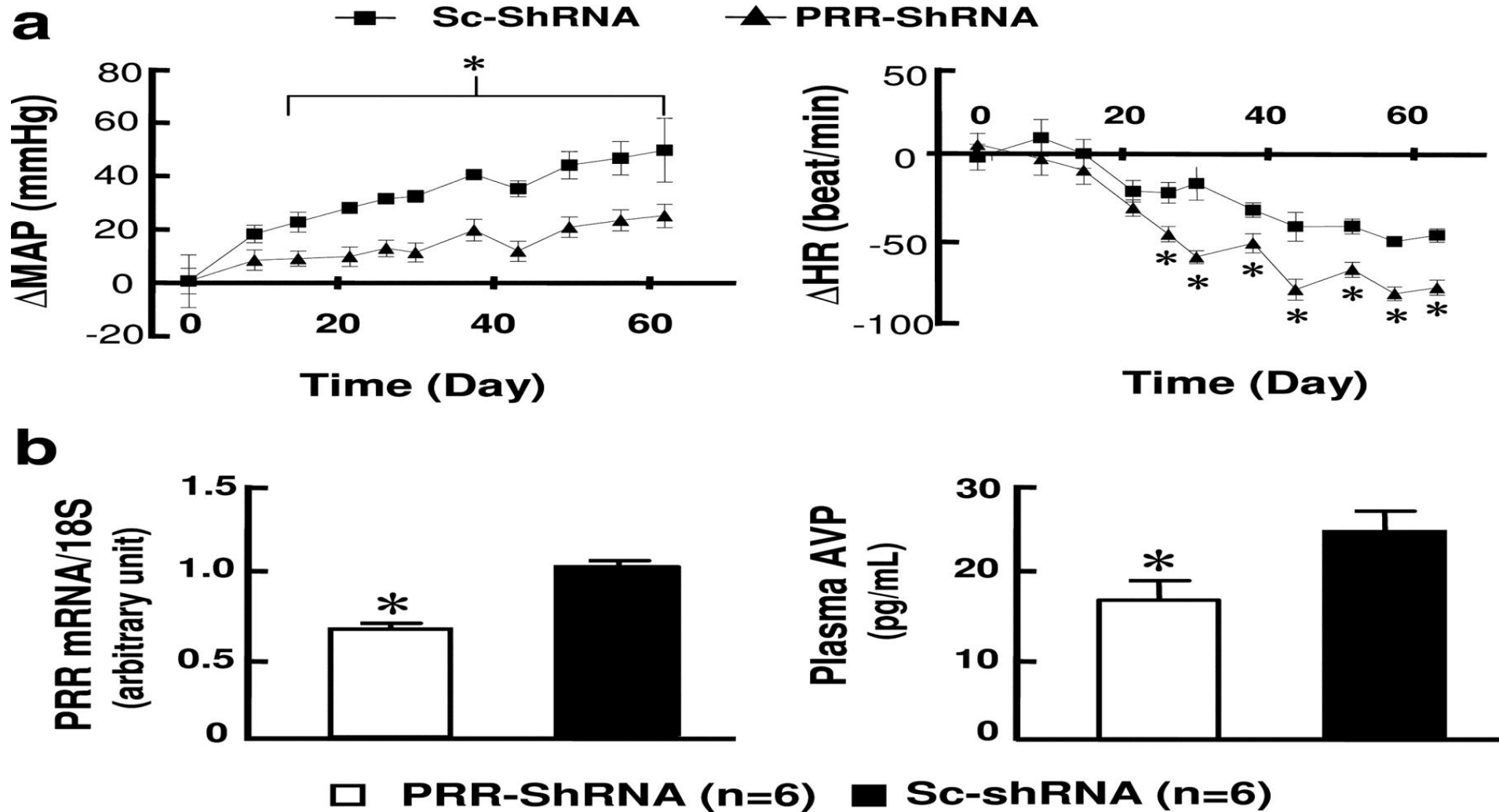
RVLM

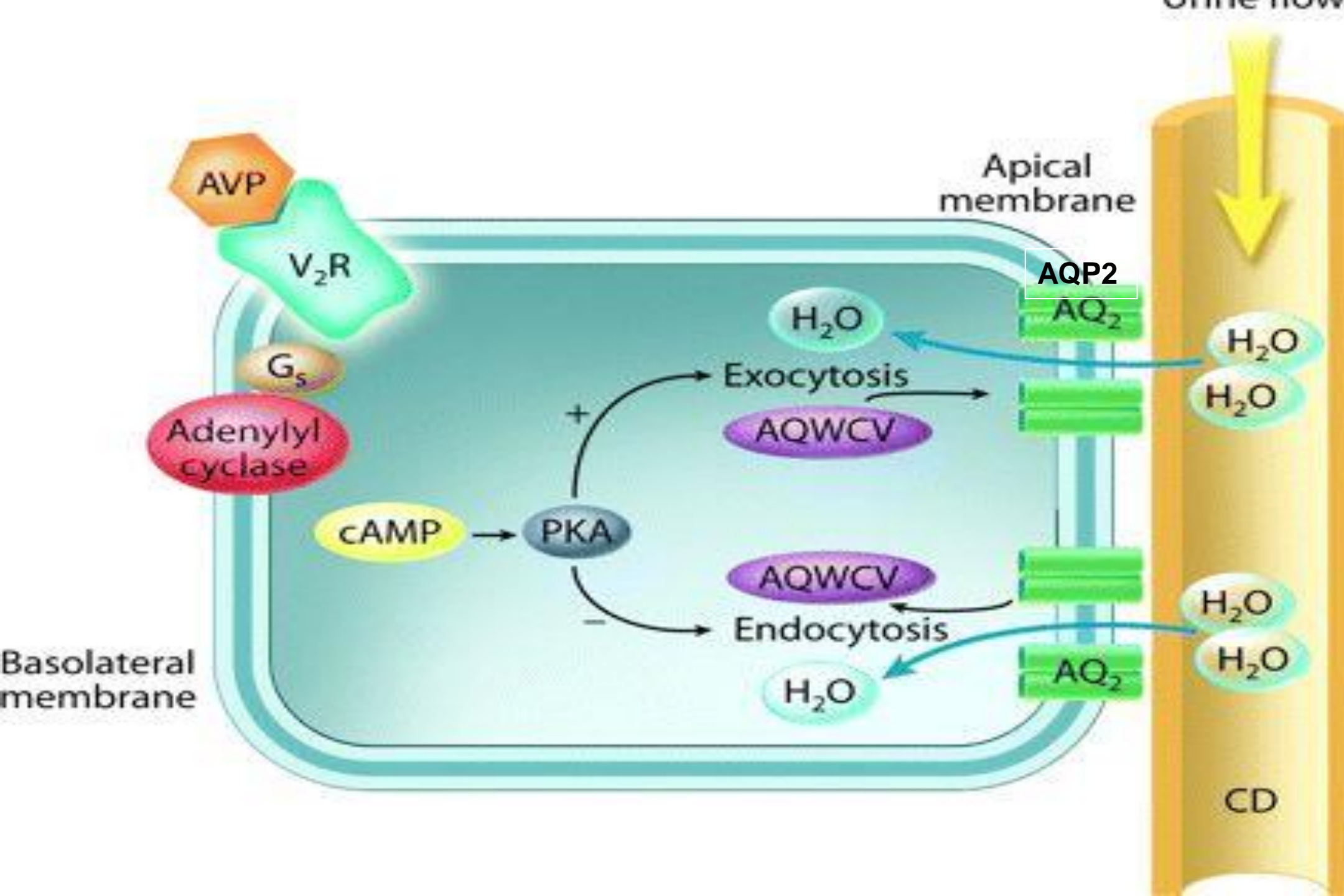


Effect of PRR overexpression in the SON of SD rats on fluid homeostasis

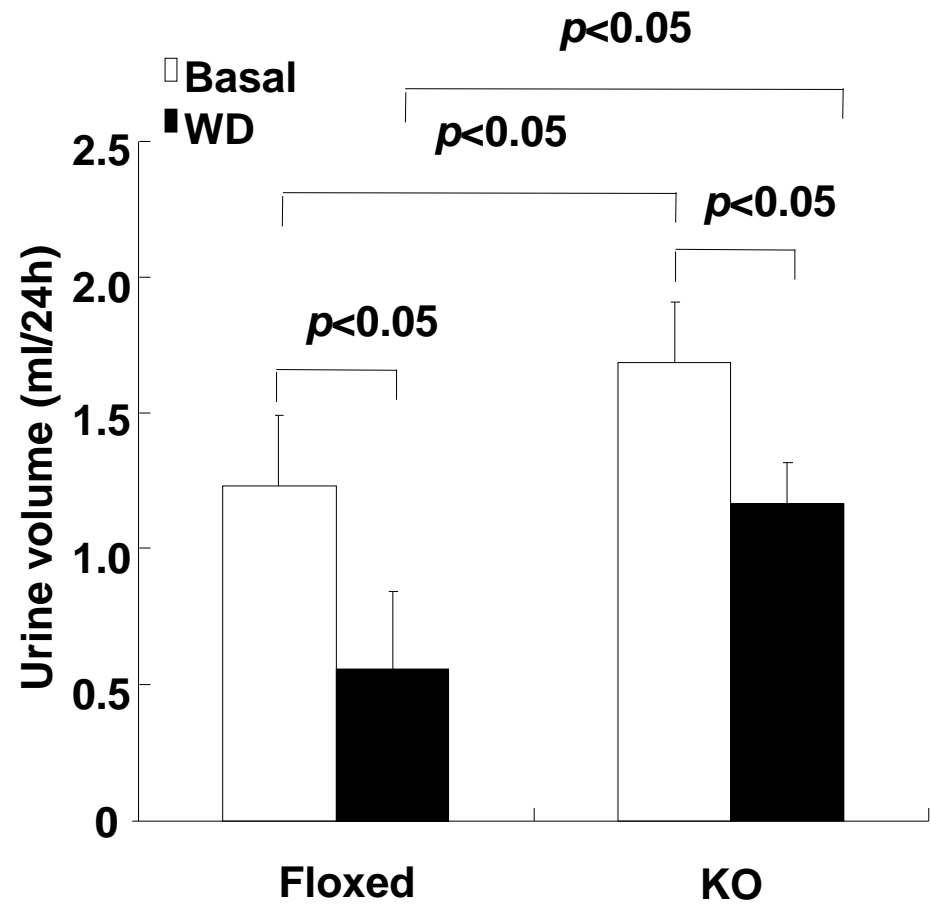
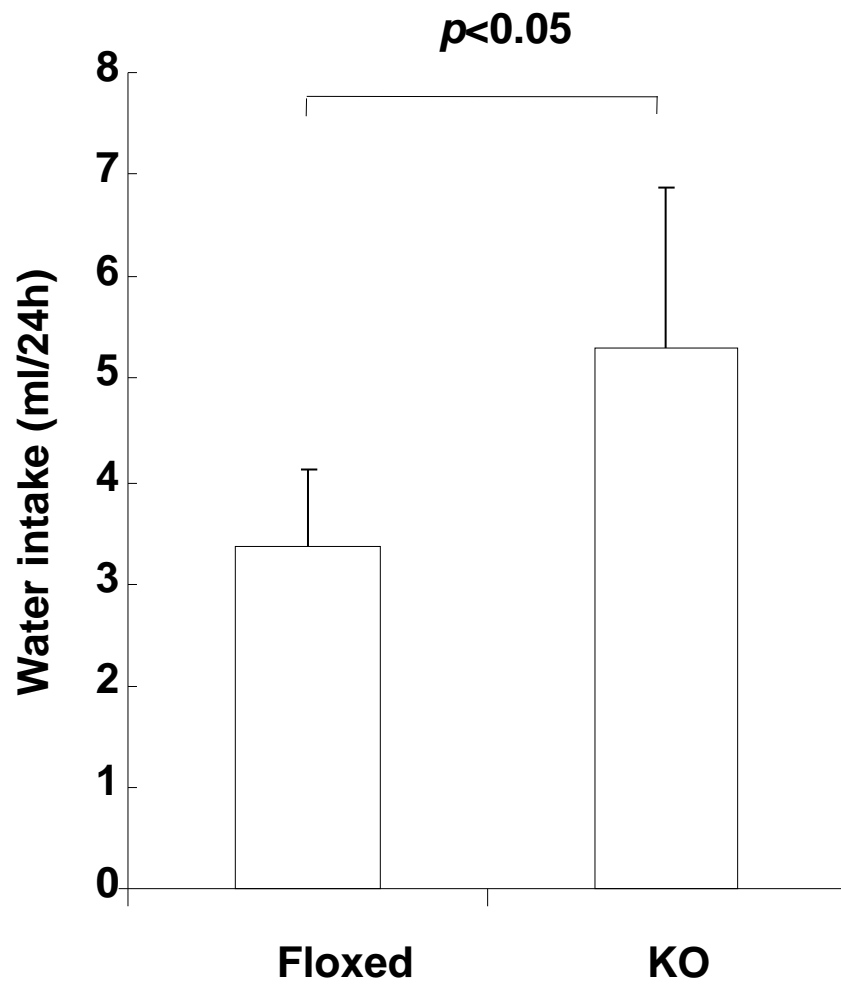


Effect of PRR knockdown in the SON of SHRs on cardiovascular function

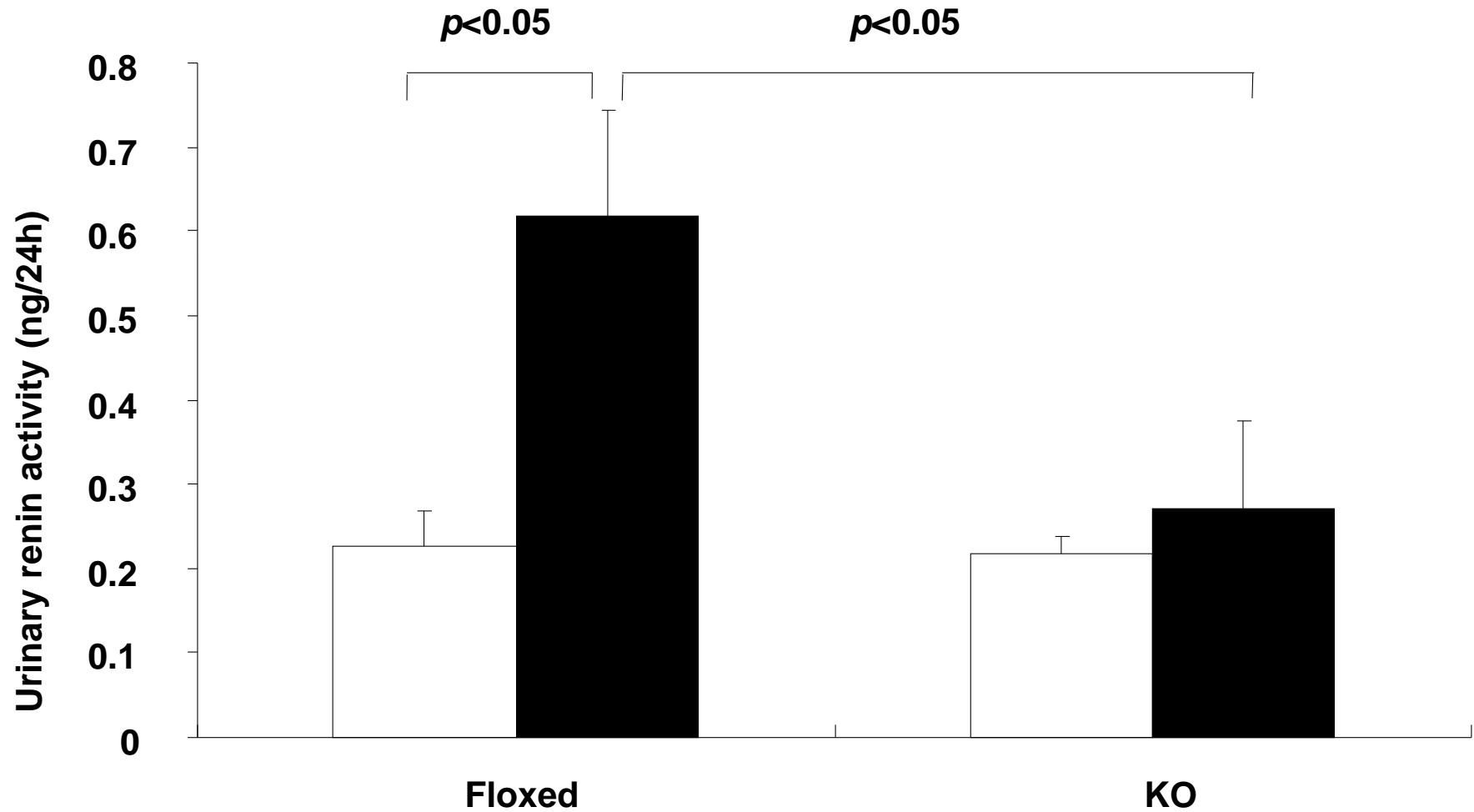




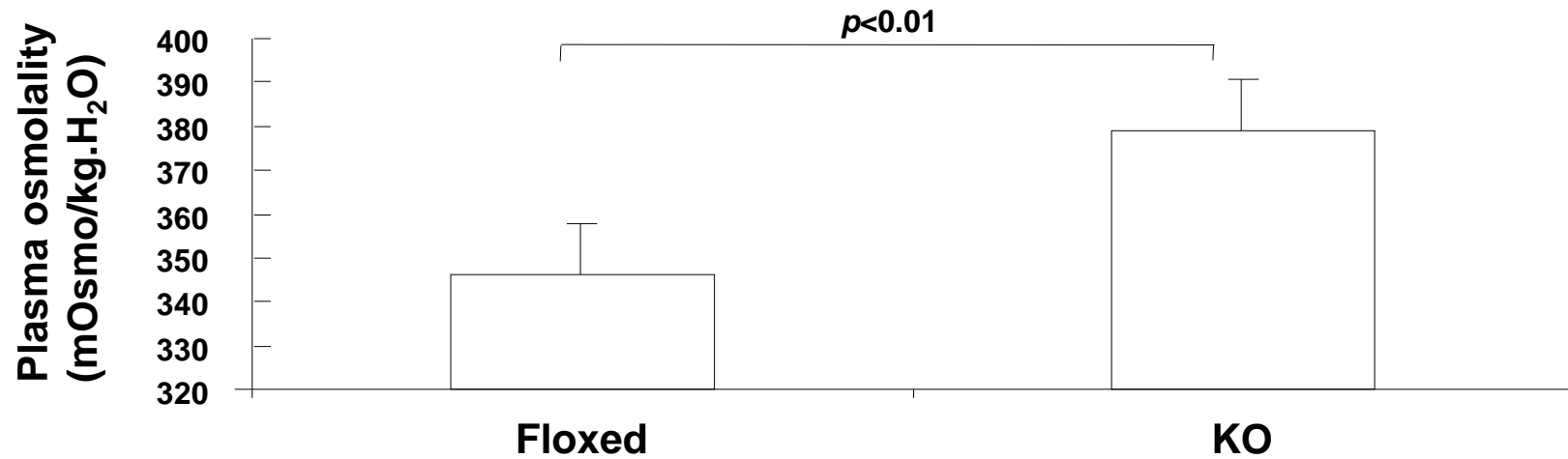
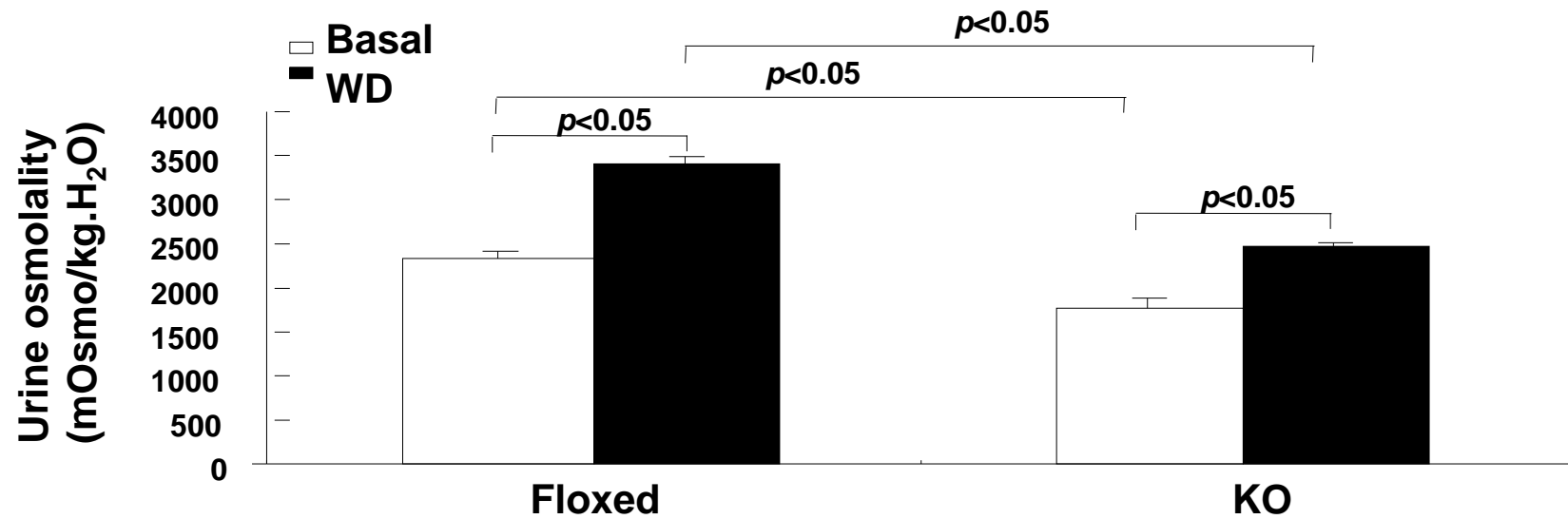
Fluid Metabolism in CD PRR KO mice



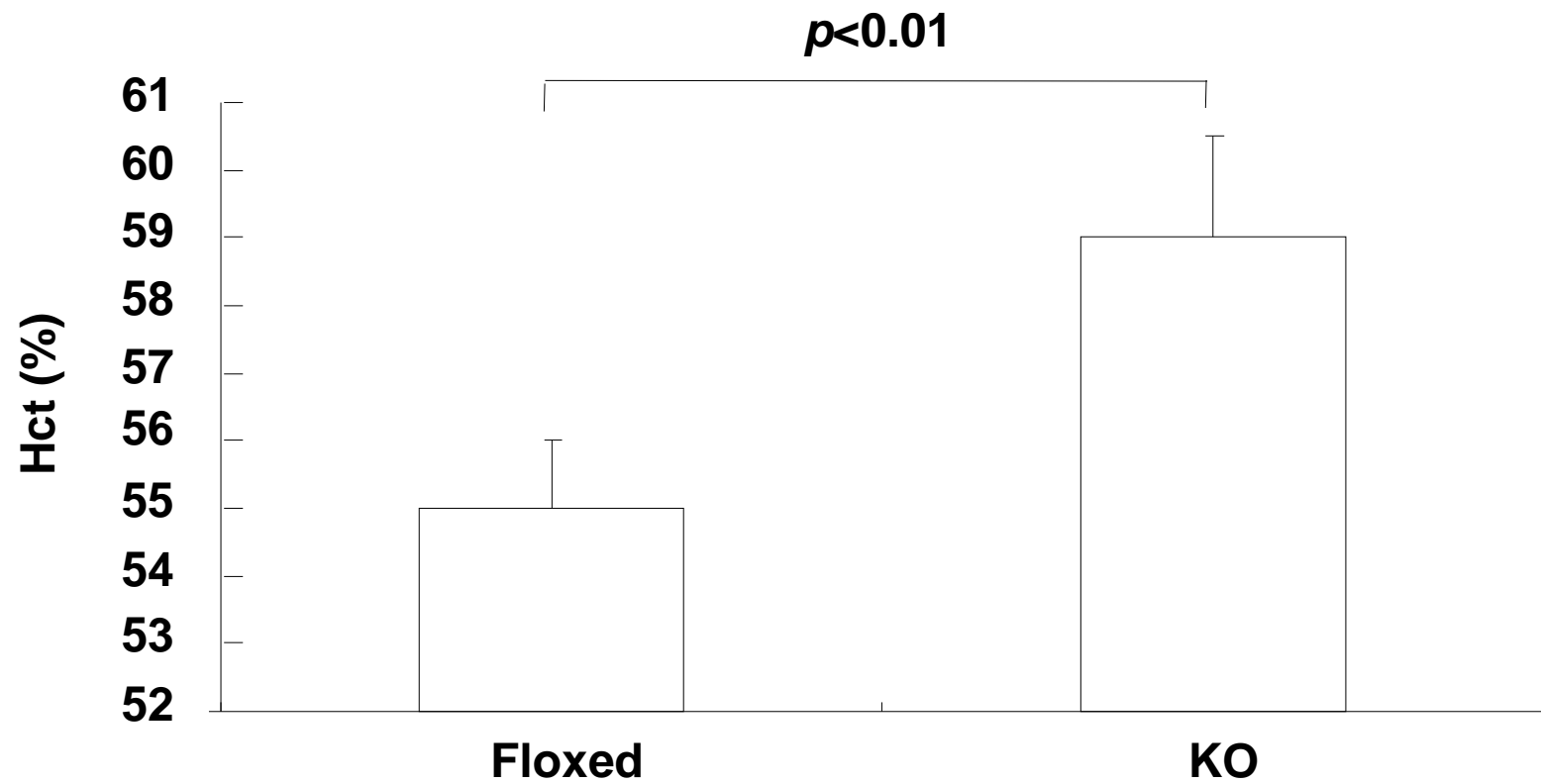
Urinary Renin Activity in CD PRR KO mice



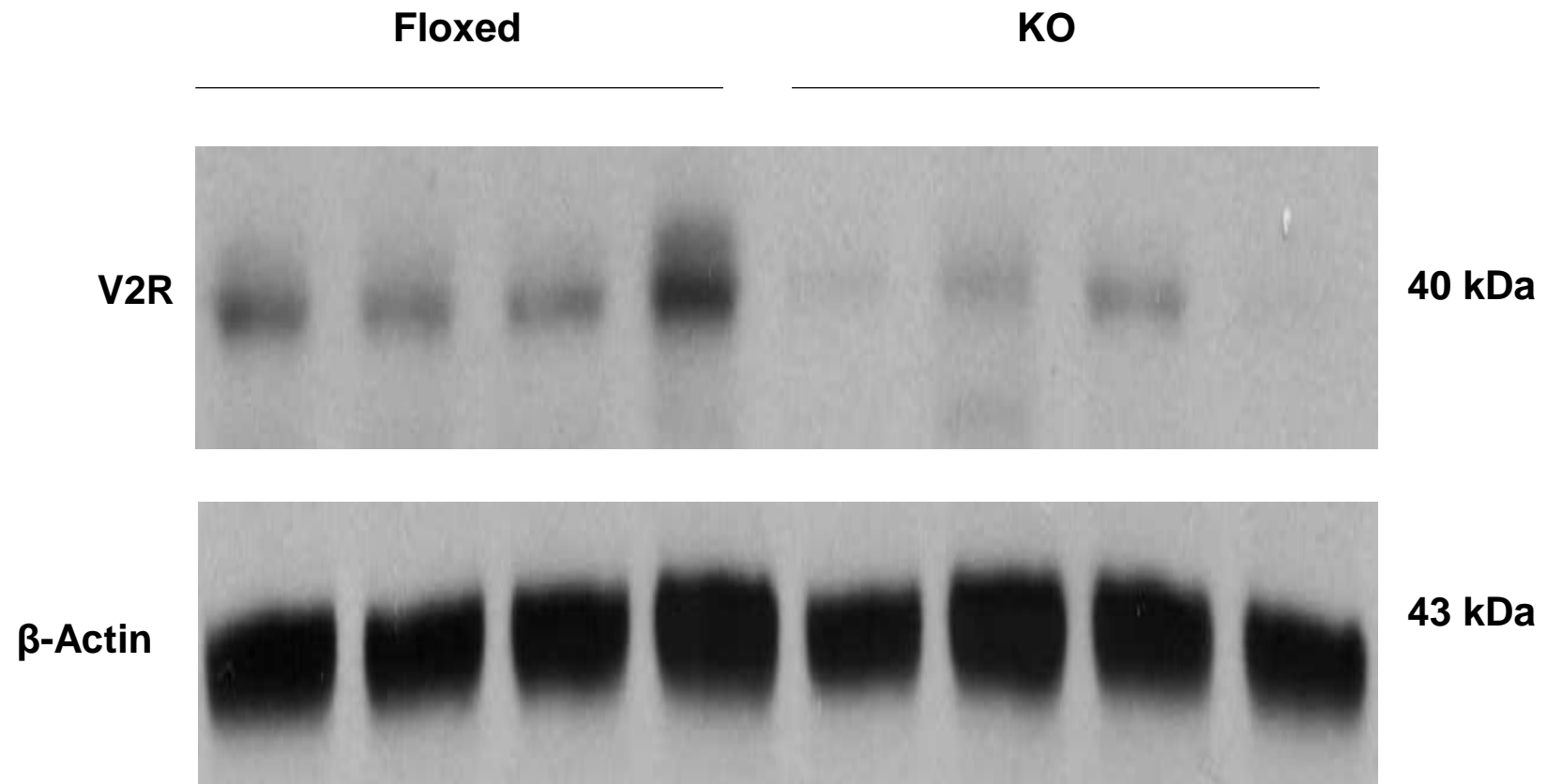
Urine and Plasma Osmolality in CD PRR KO mice



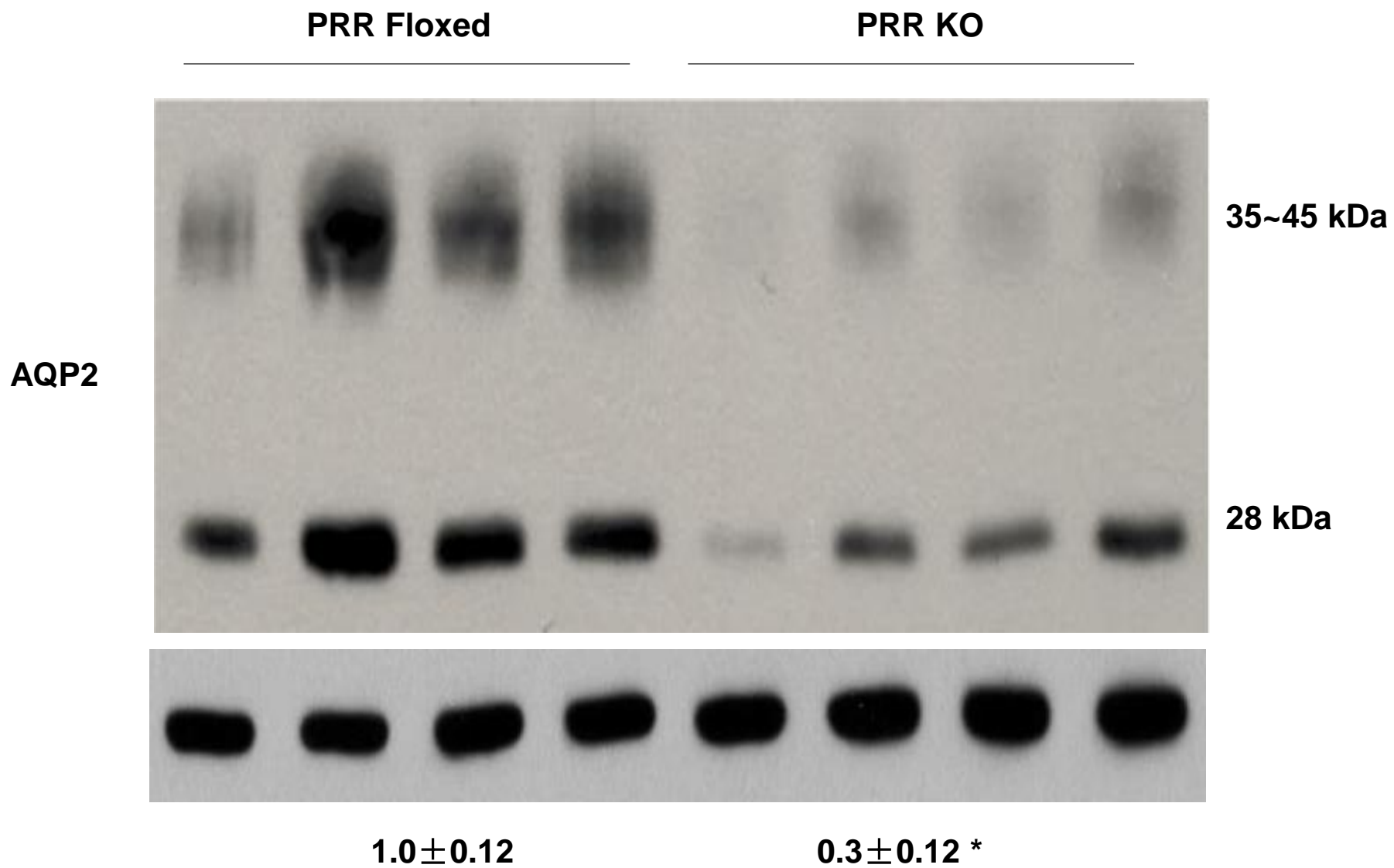
Hct in CD PRR KO Mice after 24h Water Deprivation



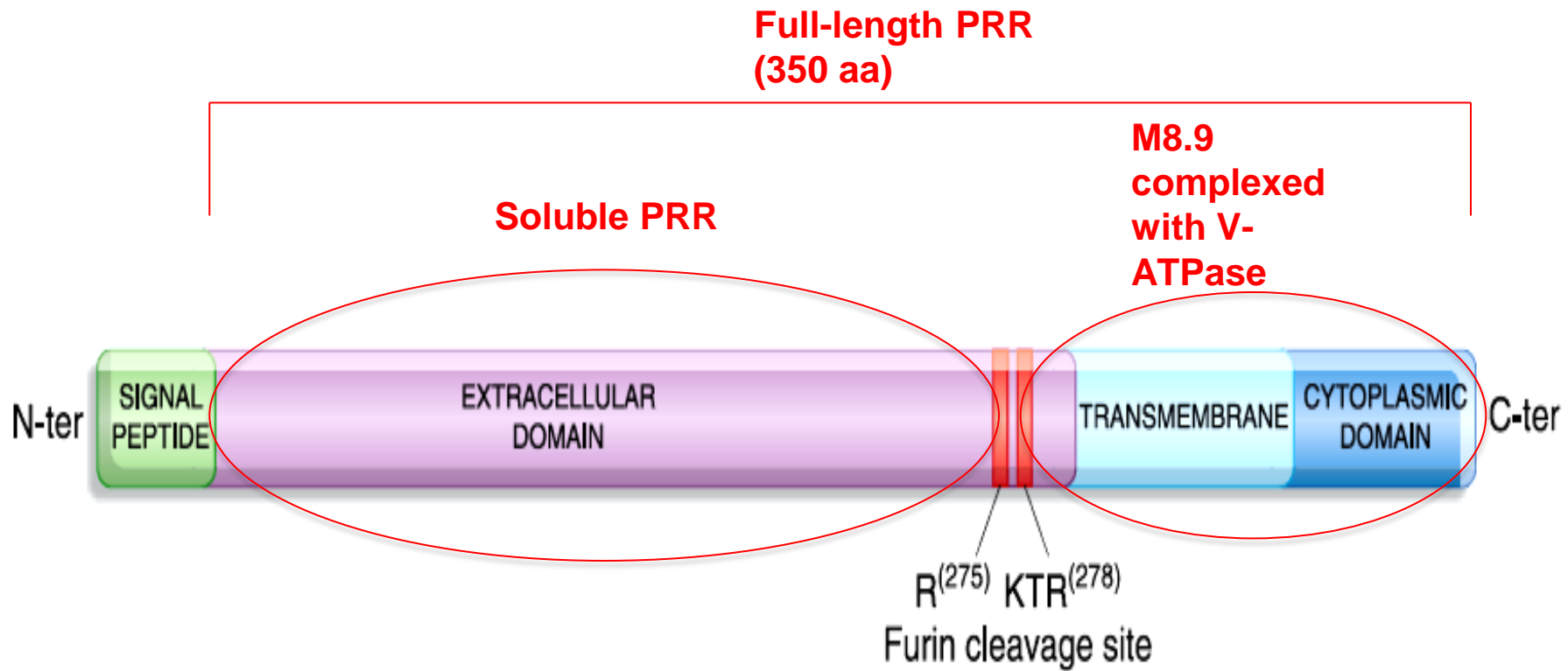
Renal Medullary V2R Protein Expression in CD PRR KO Mice



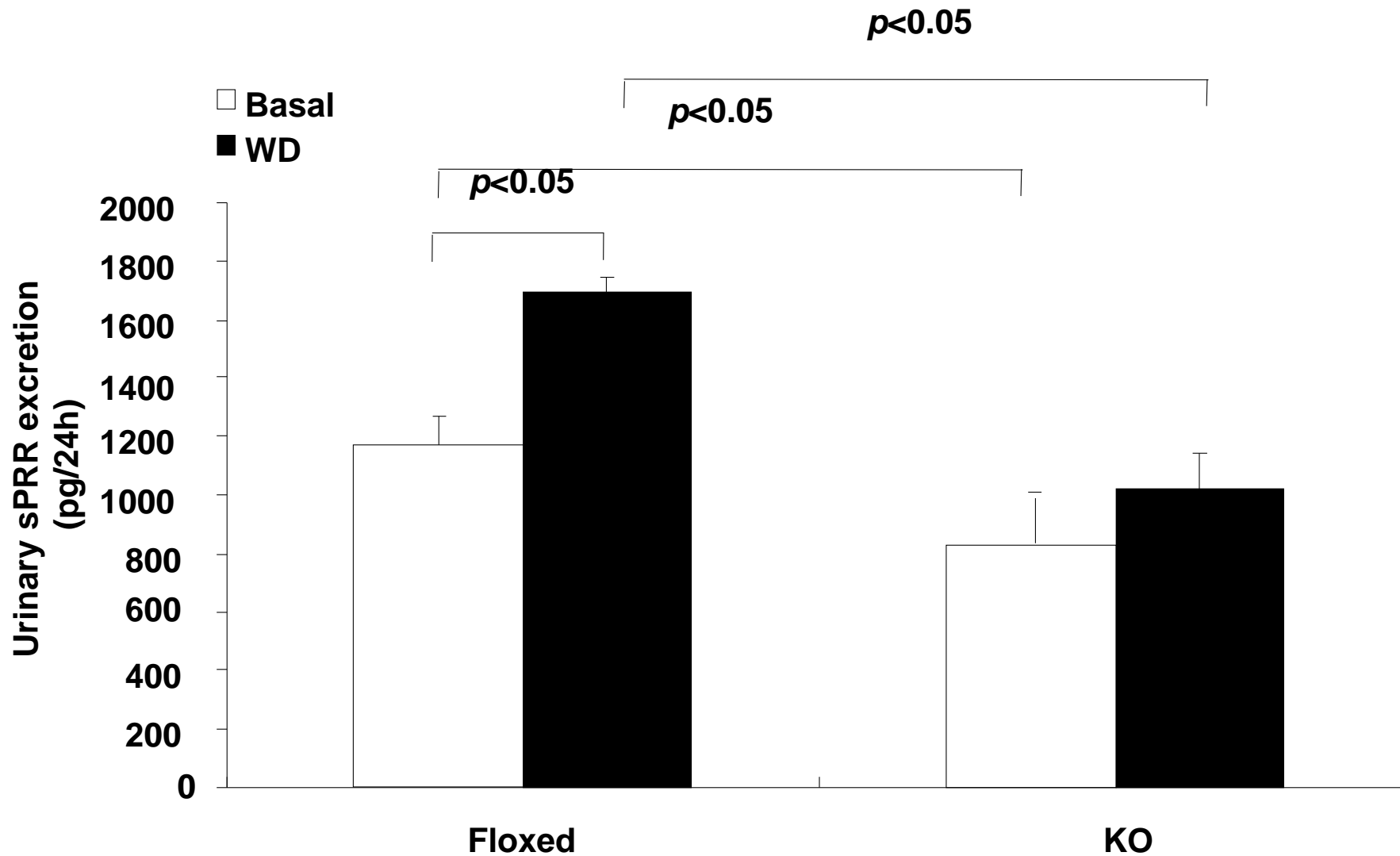
Renal Medullary AQP2 Protein Expression in CD PRR KO Mice



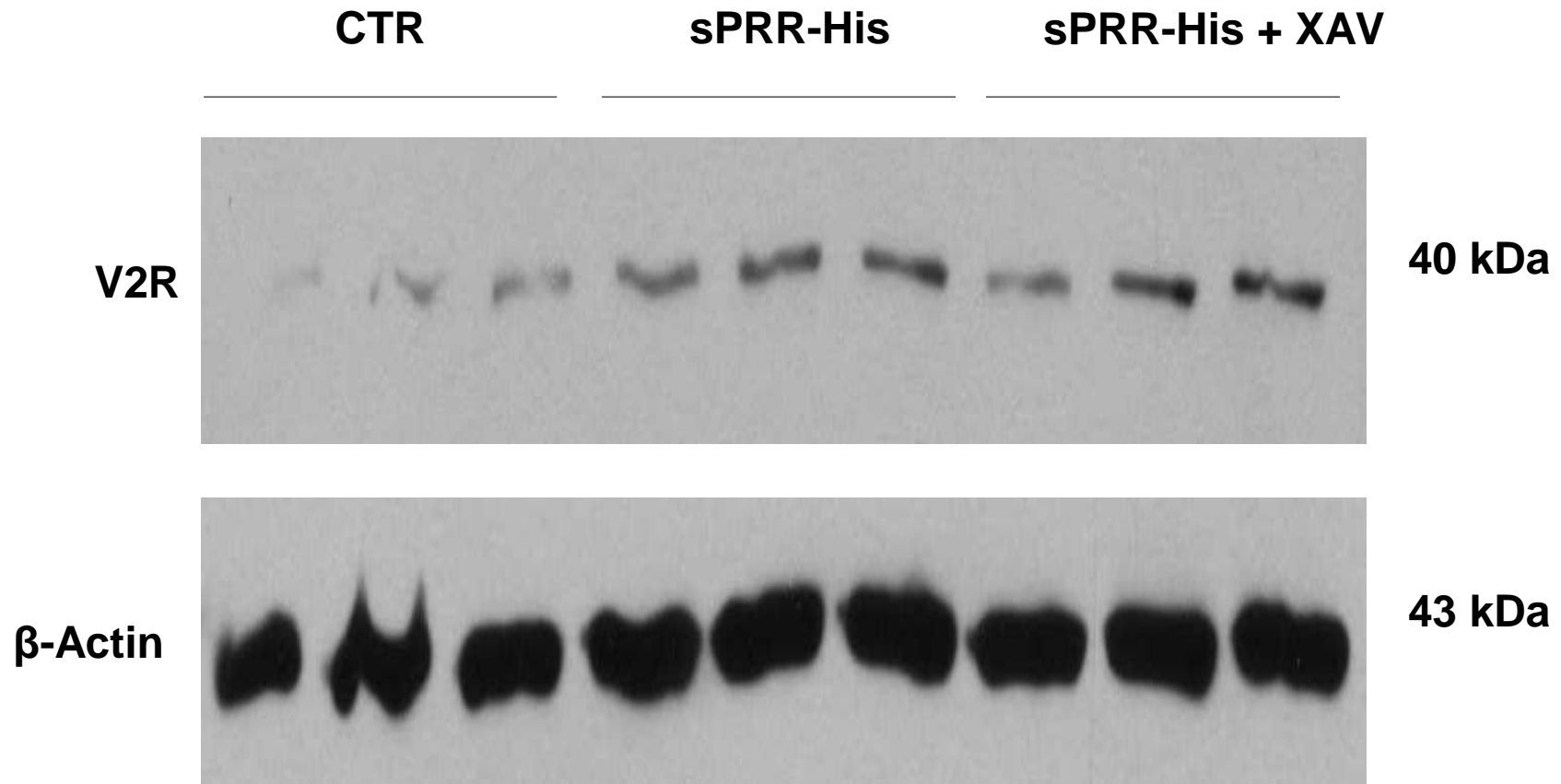
Structure of (Pro)Renin Receptor (PRR) (ATP6AP2)



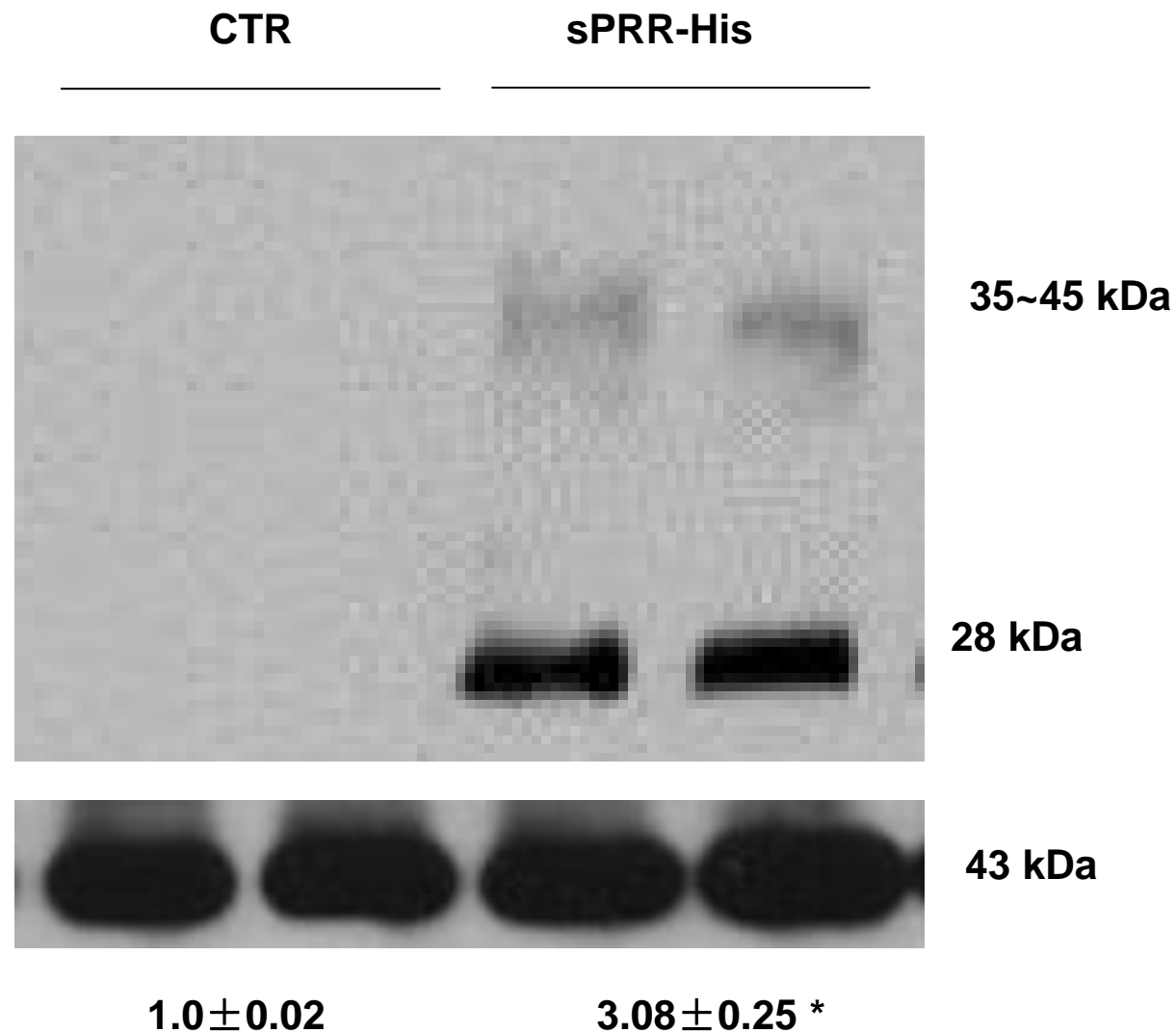
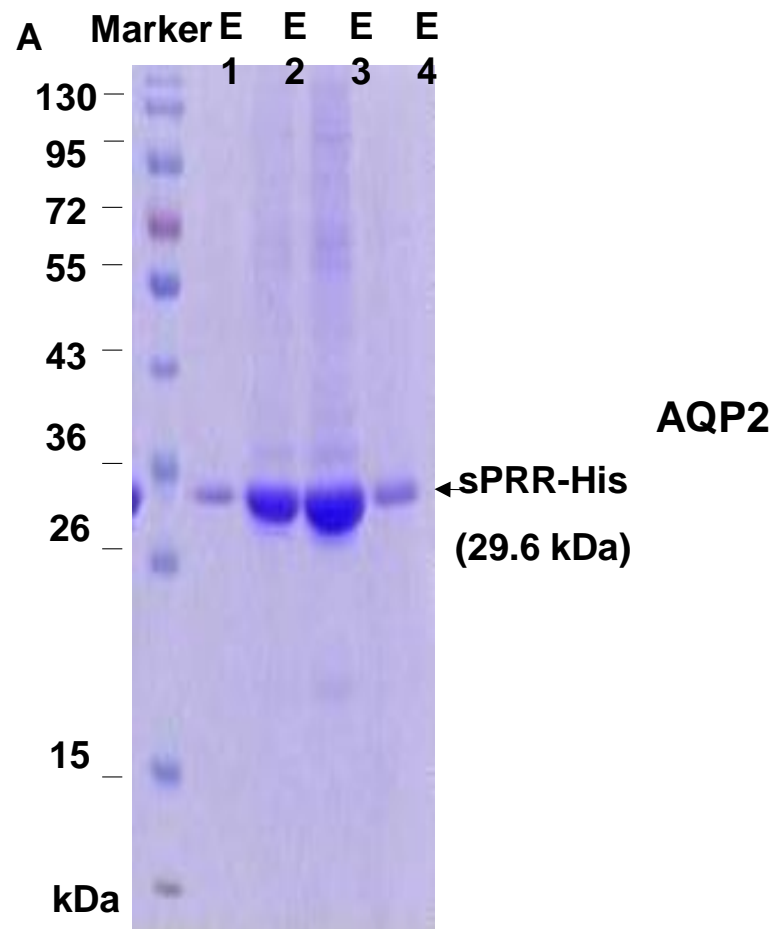
Urinary sPRR Excretion in CD PRR KO Mice



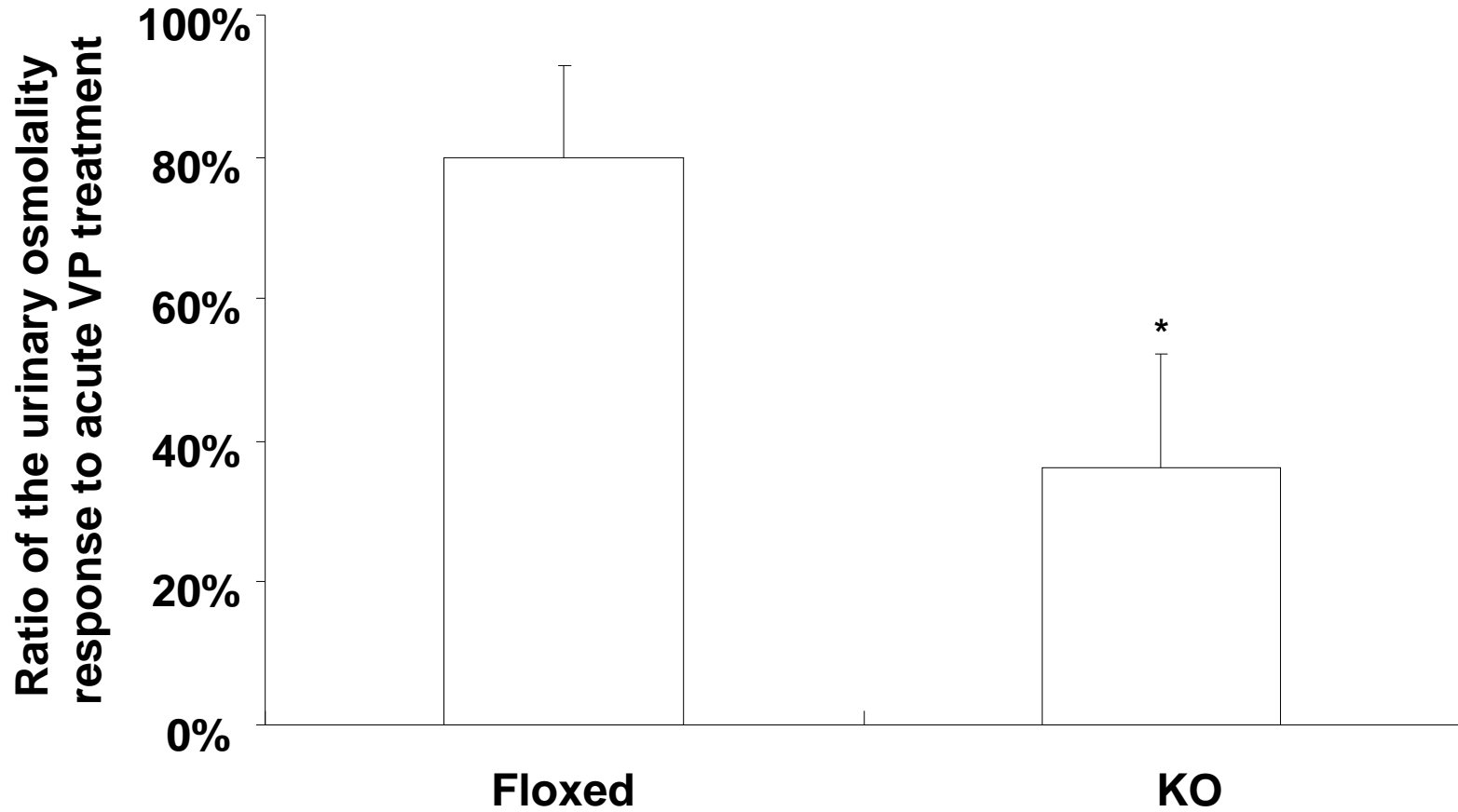
sPRR Stimulated V2R Expression in Cultured IMCD Cells



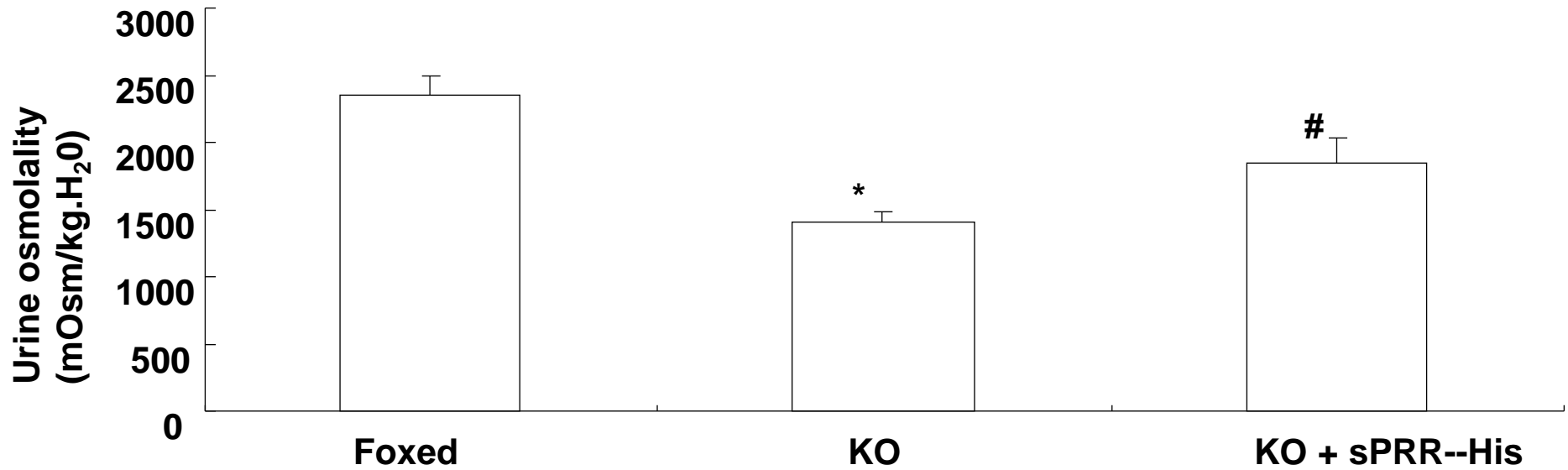
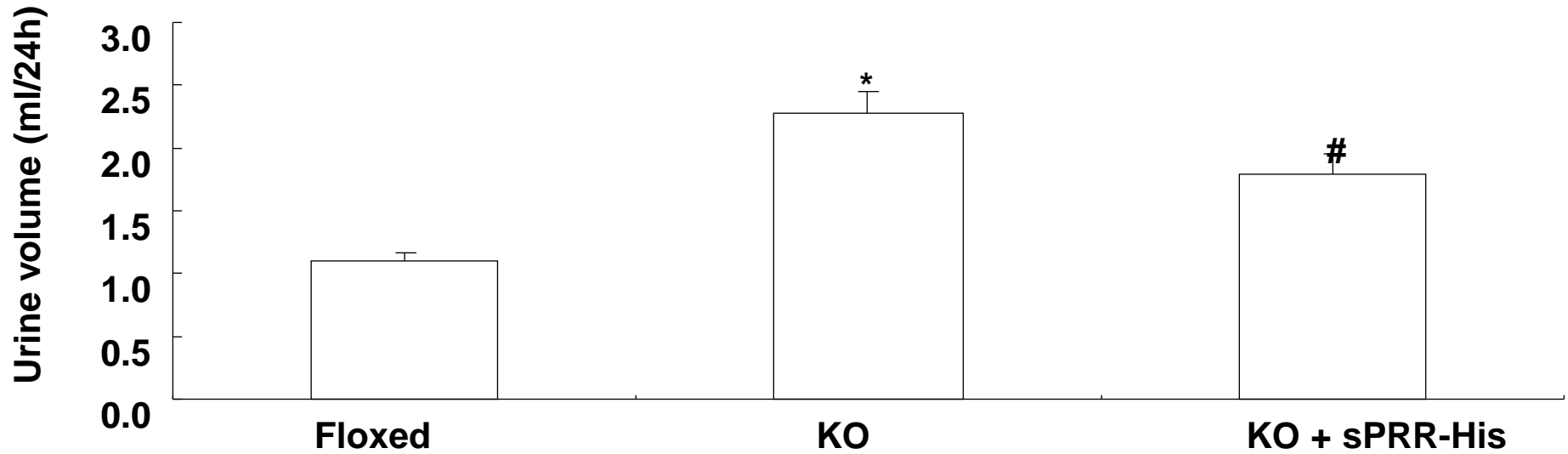
Effect of sPRR-His on AQP2 Expression in Cultured IMCD Cells



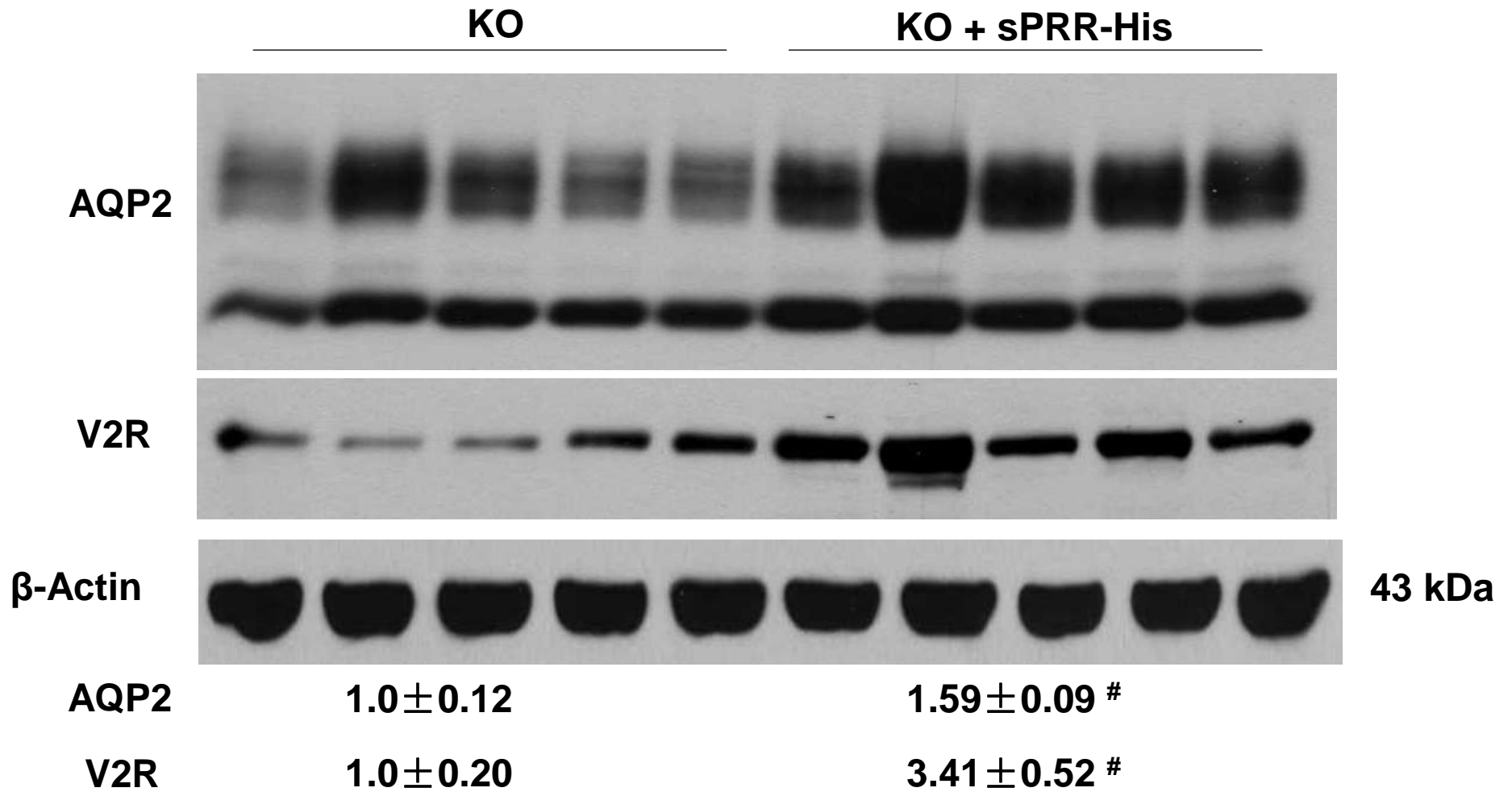
Response of Urine Osmolality to AVP



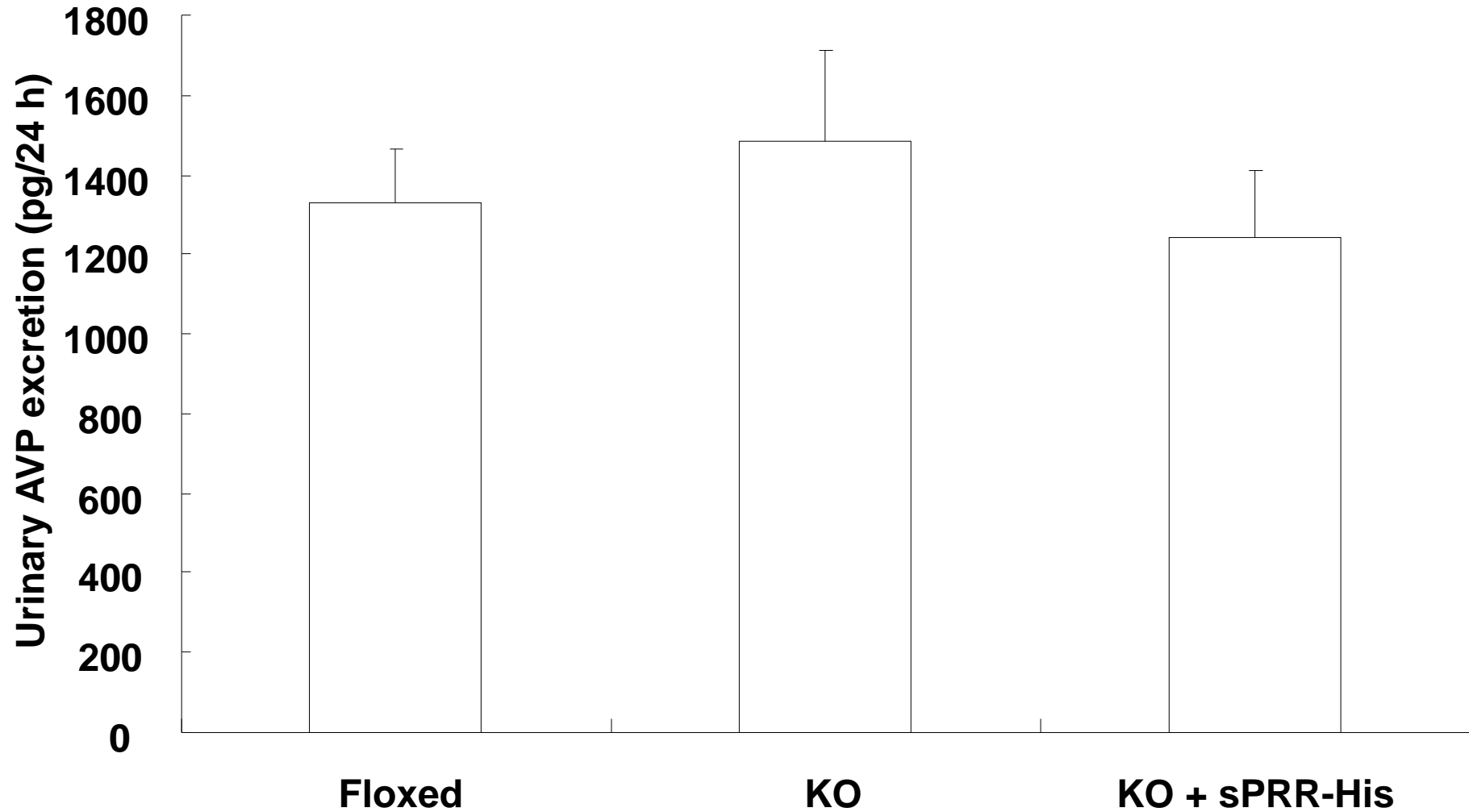
IV Infusion of sPRR-His Partially Rescues the Polyuria Phenotype of CD PRR KO Mice

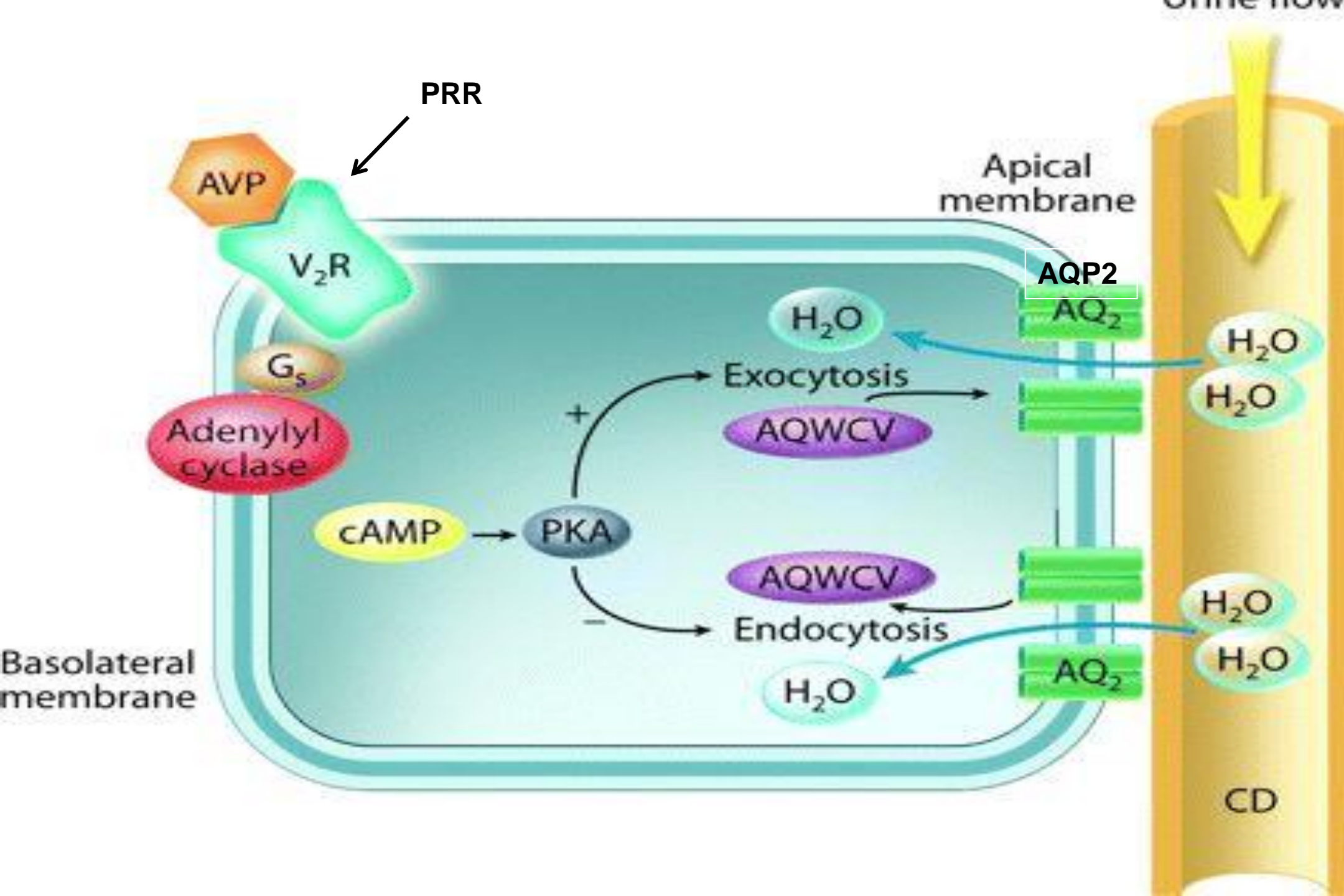


IV Infusion of sPRR-His Elevated Renal V2R and AQP2 Expression in CD PRR KO Mice



No Change in AVP Production





Summary/Conclusion

- We provide in vivo evidence for PRR as a regulator of local renin in the the kidney.
- Activation of CD PRR mediates local renin response and determines AngII-induced hypertension.
- In addition, CD PRR regulates BP and fluid balance via simultaneously controlling central AVP production and peripheral AVP sensitivity.
- The action of CD PRR is mediated by sPRR .

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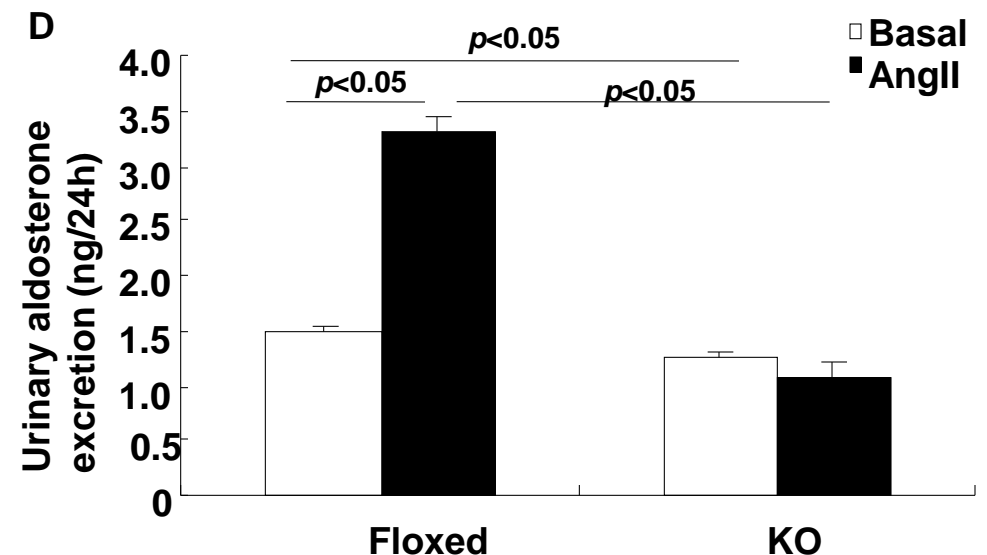
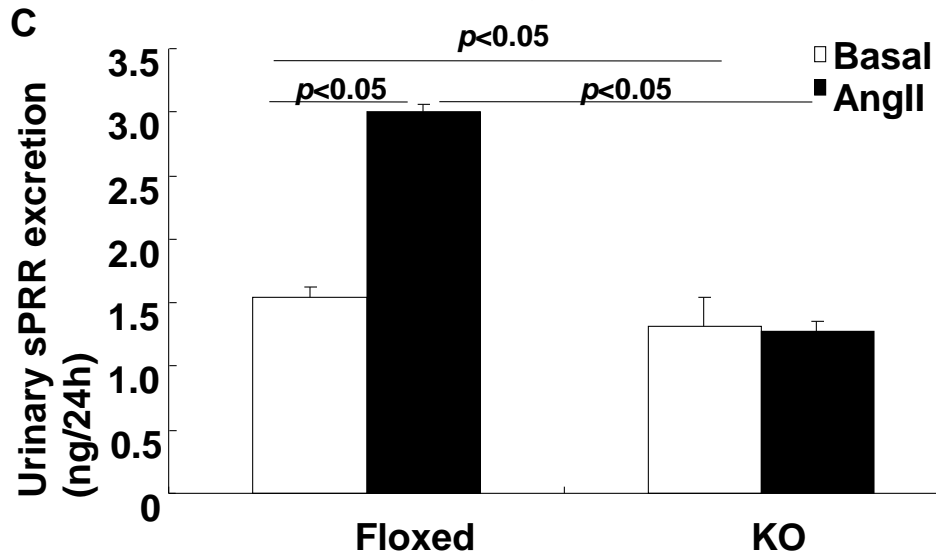
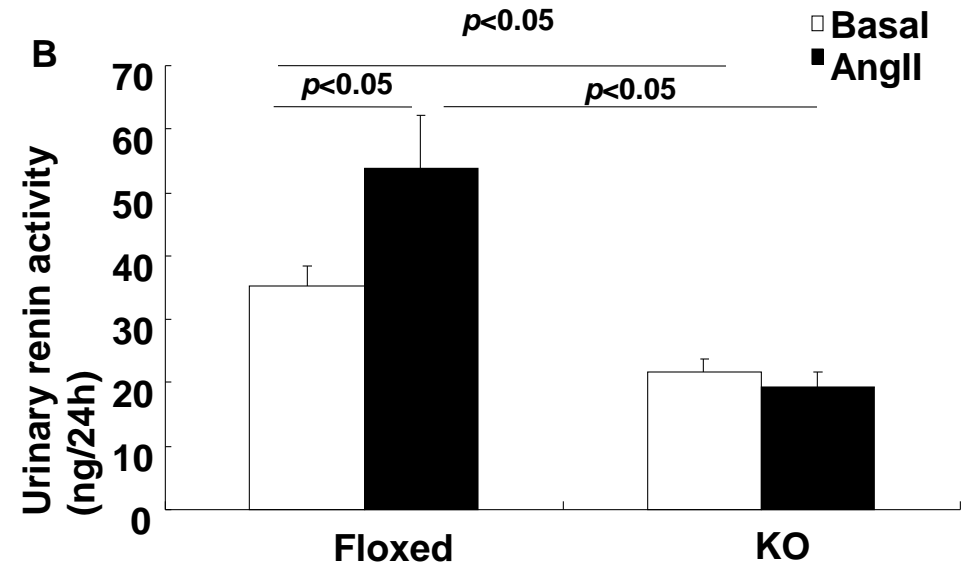
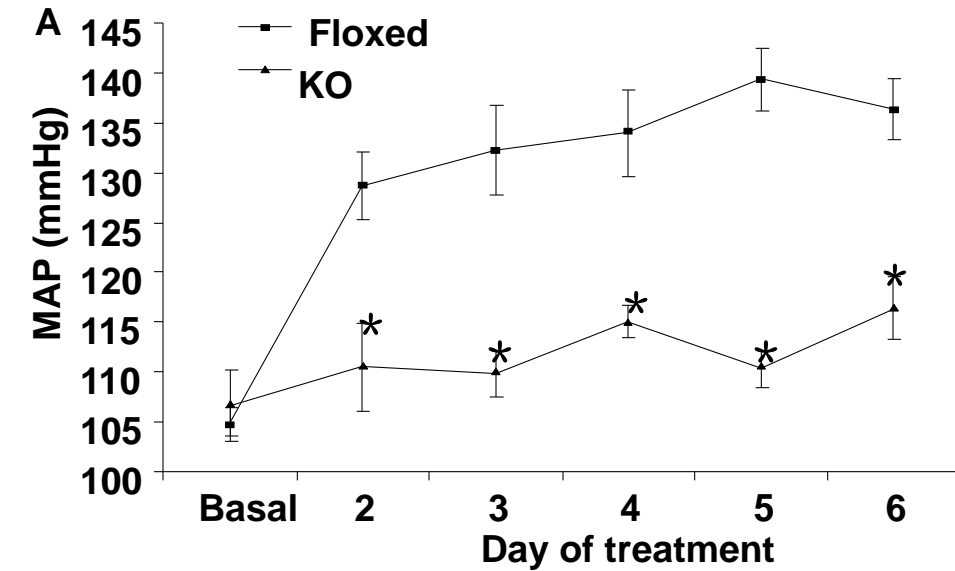
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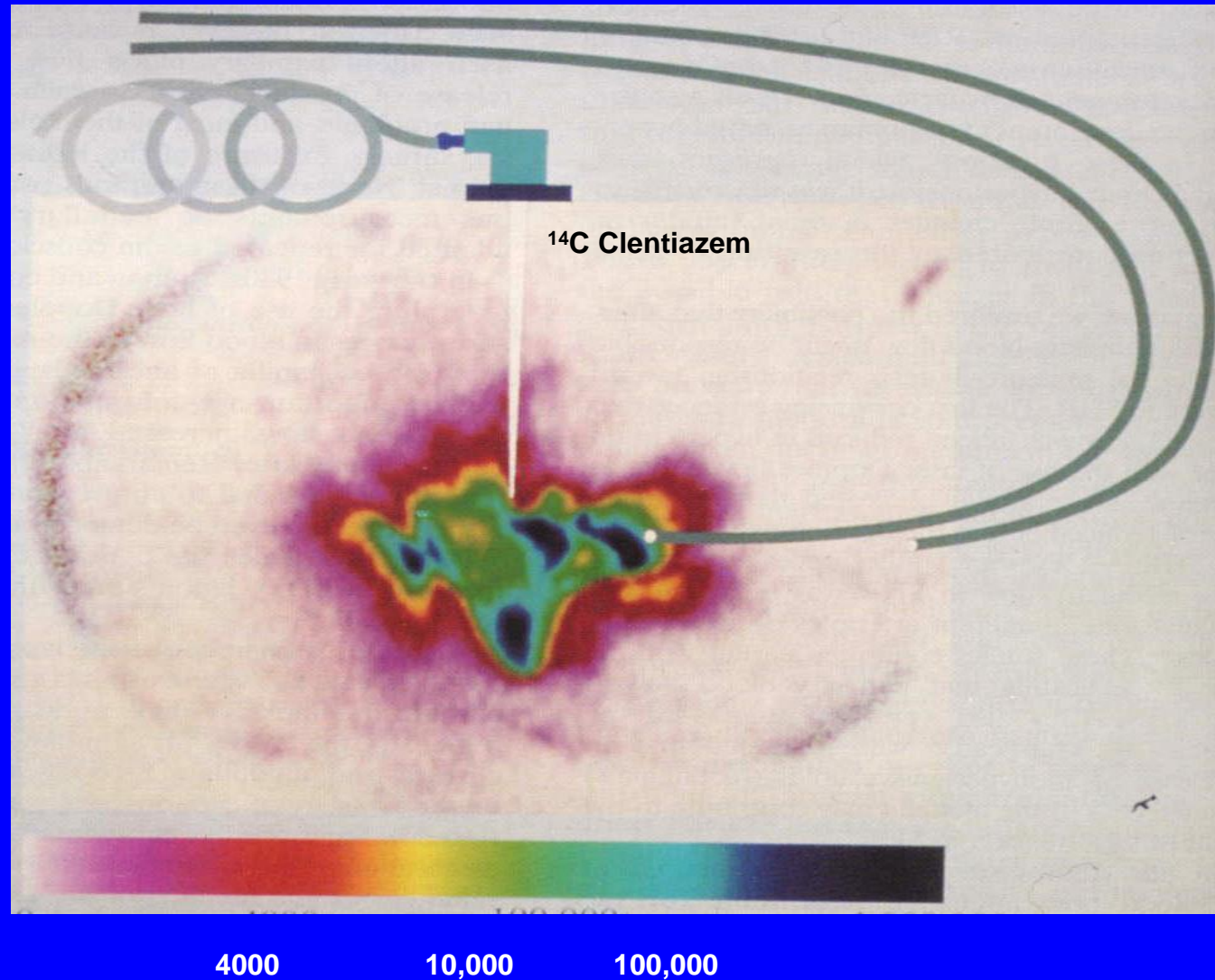
NSFC

973 Sub-project

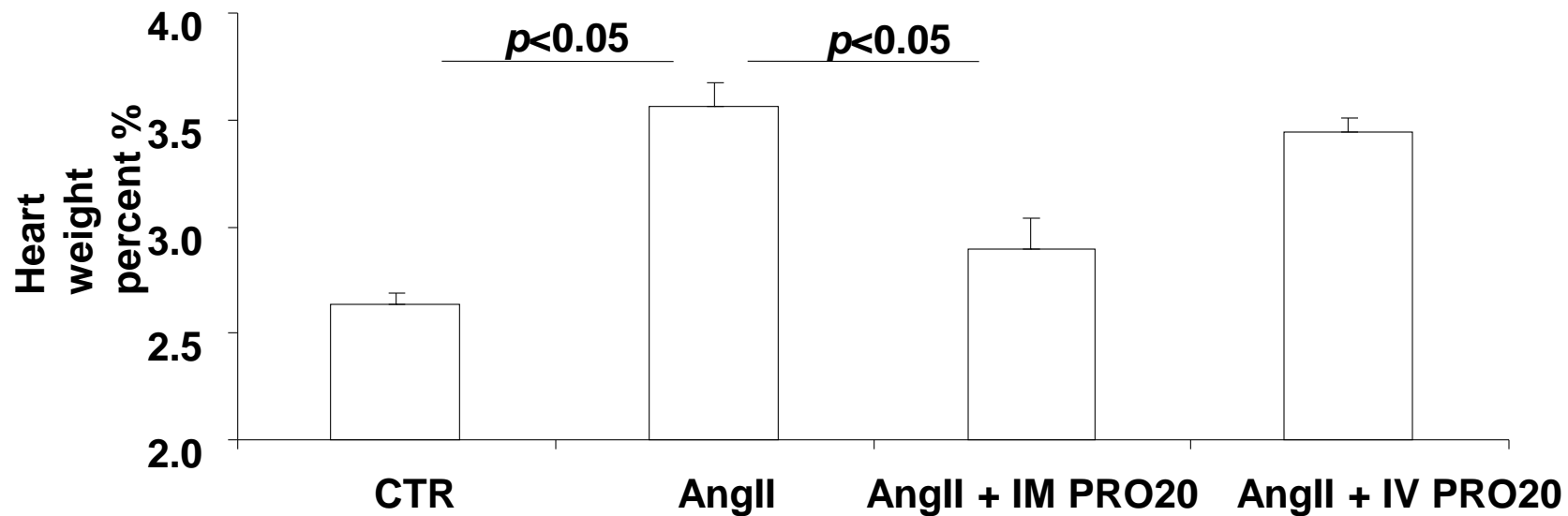
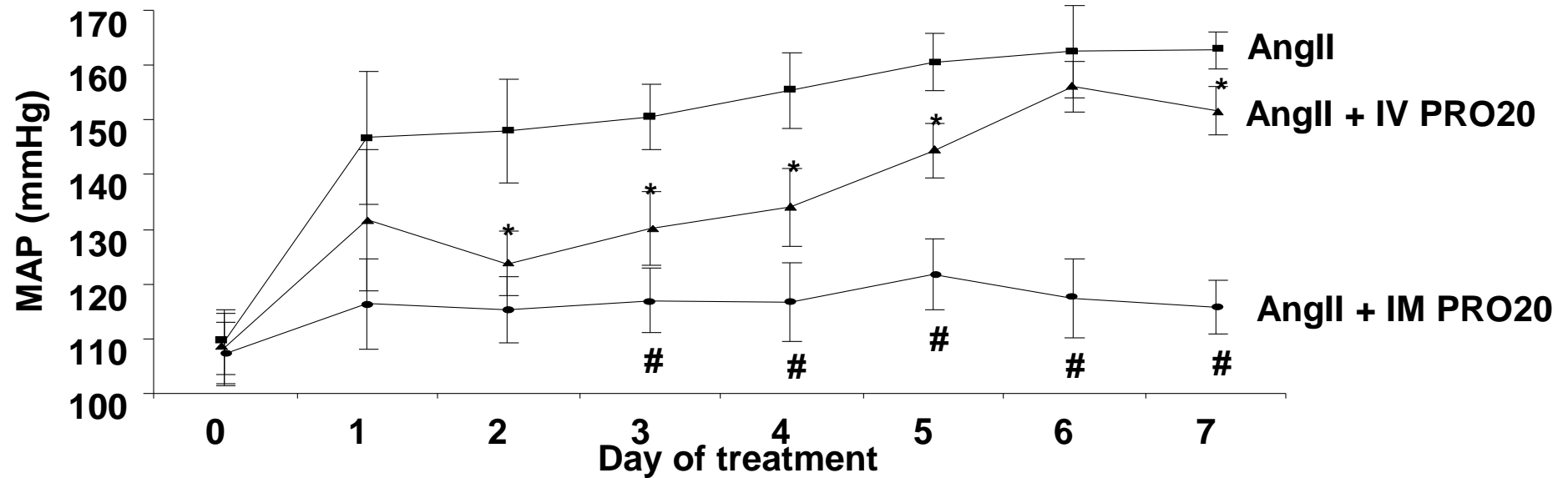
Phenotypical Analysis of CD PRR KO mice after AngII Infusion



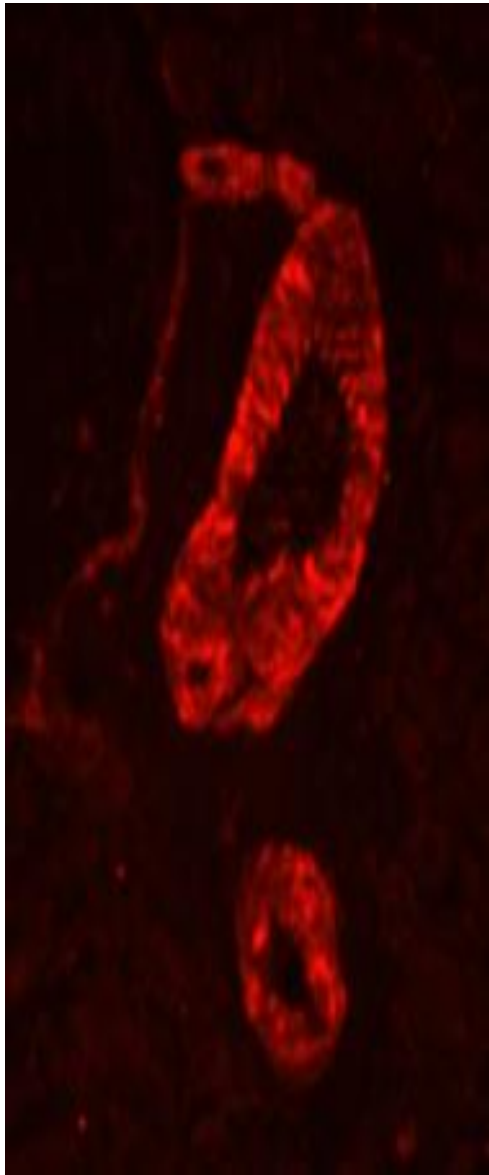
Intramedullary Infusion Technique



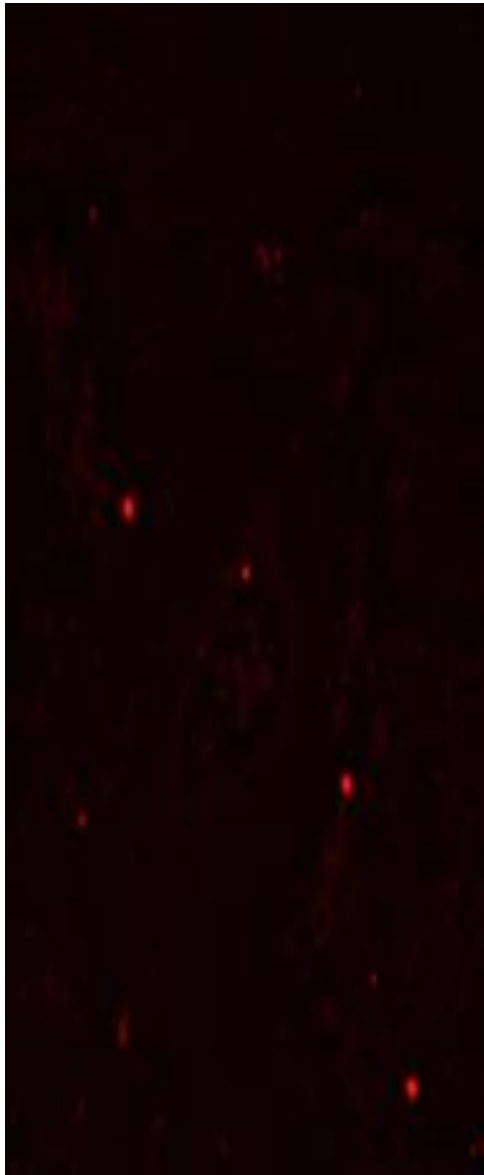
MAP in Rats Receiving Intramedullary or Intravenous Infusion of PRO20



PRR - kidney



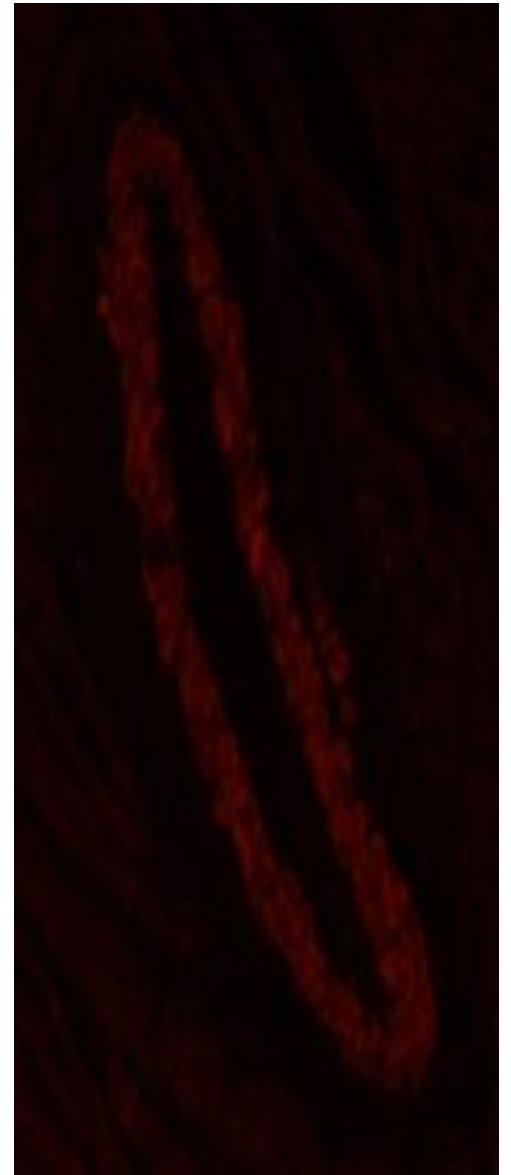
PRR+peptide



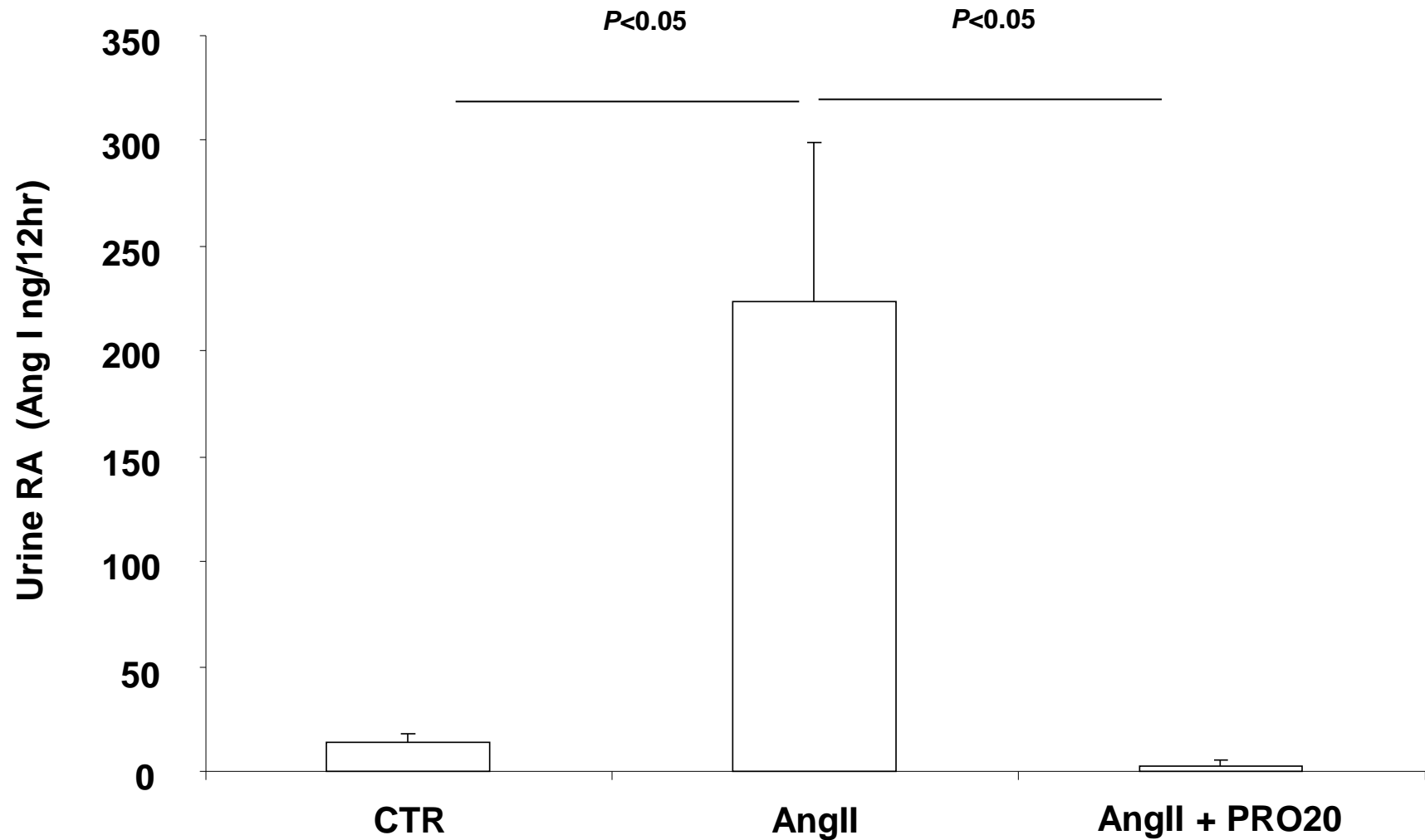
IgG



PRR - heart



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



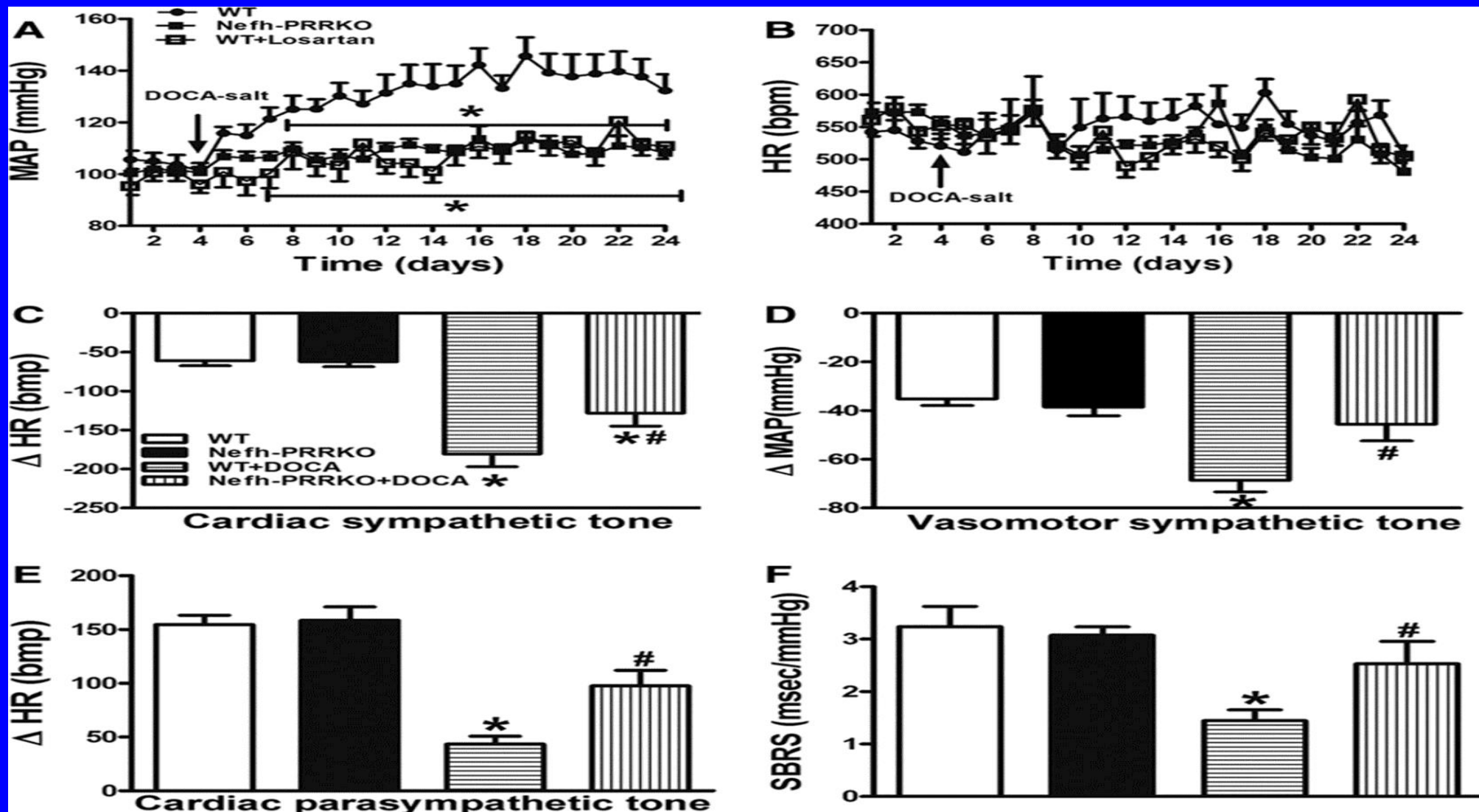
Central PRR

- Within the CNS, PRR is predominantly expressed in cardiovascular-relevant neurons including the paraventricular (PVN) and supraoptic nuclei (SON) and the subfornical organ (SFO) (Shan Z et al. 2010; Takahashi K et al. 2010)
- PRR is co-localized with vasopressin (AVP) and oxytocin in SON and PVN in humans (Takahashi K et al. 2010)
- PRR knockdown in the SON of spontaneously hypertensive rats (SHR) decreases MAP and heart rate and plasma AVP level (Shan Z et al. 2010)
- Conversely, in the same study, targeted overexpression of human PRR in the SON of Wistar-Kyoto (WKY) rats induced a 2-fold increase in plasma AVP and a 3-fold increase in urinary AVP, in parallel with enhancement of urine concentrating capability (Shan Z et al. 2010)
- Neuron-specific deletion of PRR or intracerebroventricular (ICV) infusion of a PRR decoy inhibitor PRO20 attenuates the hypertension induced by AngII or DOCA-salt (Li W et al. 2012; Li W et al. 2014; Li W et al. 2014).

Evidence Arguing against PRR as a Renin Regulator

- Overexpression of human PRR in rats resulted in proteinuria and nephropathy but did not elevate BP or renal AngII levels (Kaneshiro Y 2007)
- Challenges: Lack of viable KO; controversial handle region peptide (HRP).

Neuron-Specific PRR Deletion Attenuates DOCA-Salt-Induced hypertension in Mice



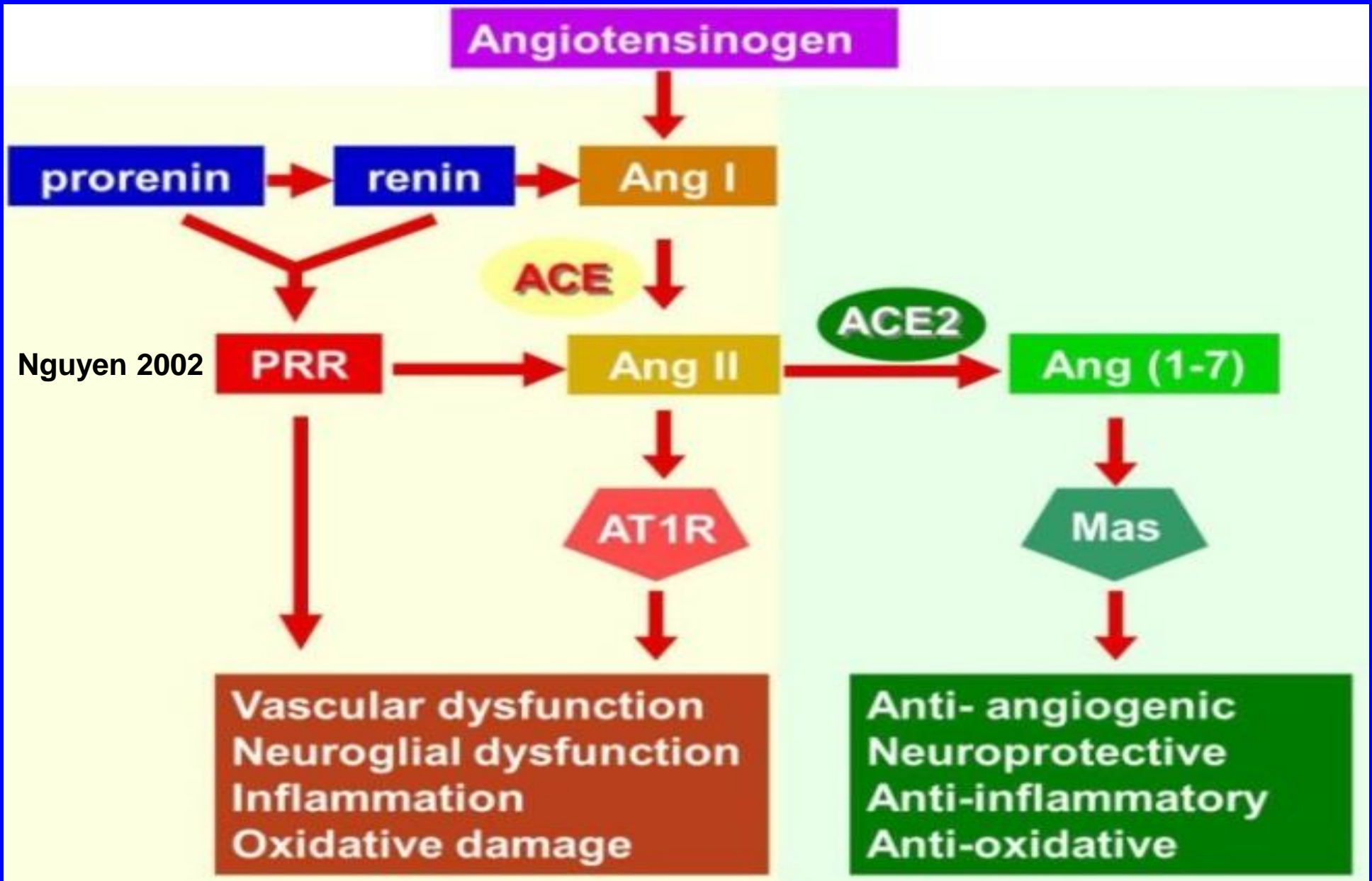
Activation of the (pro)renin receptor in the paraventricular nucleus increases sympathetic outflow in anesthetized rats

Michael J. Huber, Rupsa Basu, Cassie Cecchetti, Adolfo E. Cuadra, Qing-Hui Chen, Zhiying Shan

American Journal of Physiology - Heart and Circulatory Physiology Published 1 September 2015 Vol. 309 no. 5, H880-H887 DOI: 10.1152/ajpheart.00095.2015

Abstract

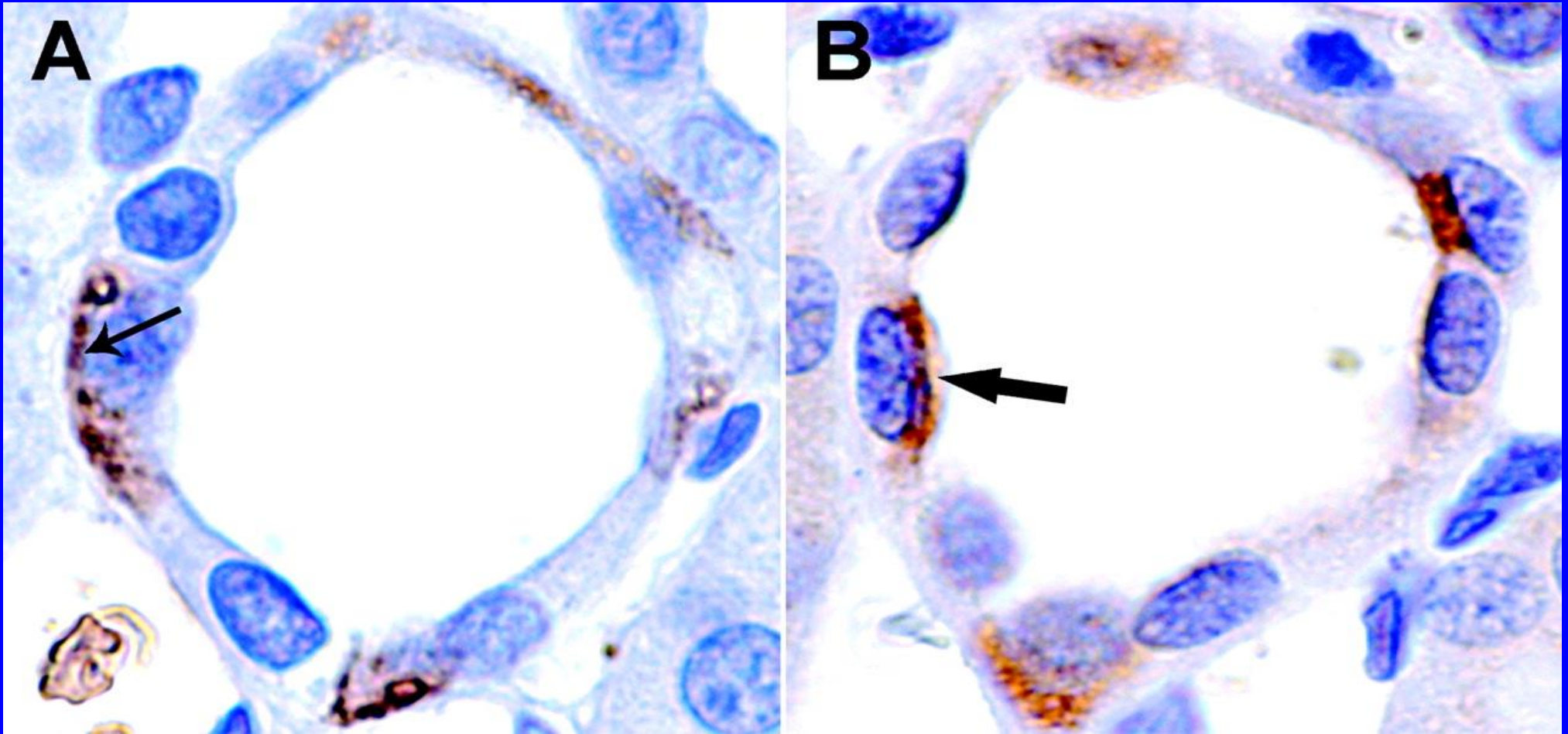
Previous studies have indicated that hyperactivity of brain prorenin receptors (PRR) is implicated in neurogenic hypertension. However, the role of brain PRR in regulating arterial blood pressure (ABP) is not well understood. Here, we test the hypothesis that PRR activation in the hypothalamic paraventricular nucleus (PVN) contributes to increased sympathetic nerve activity (SNA). In anaesthetized adult Sprague-Dawley (SD) rats, bilateral PVN microinjection of human prorenin (2 pmol/side) significantly increased splanchnic SNA (SSNA; $71 \pm 15\%$, $n = 7$). Preinjection of either prorenin handle region peptide, the PRR binding blocker (PRRB), or tiron (2 nmol/side), the scavenger of reactive oxygen species (ROS), significantly attenuated the increase in SSNA (PRRB: $32 \pm 5\%$ vs. control, $n = 6$; tiron: $8 \pm 10\%$ vs. control, $n = 5$; $P < 0.05$) evoked by prorenin injection. We further investigated the effects of PRR activation on ROS production as well as downstream gene expression using cultured hypothalamus neurons from newborn SD rats. Incubation of brain neurons with human prorenin (100 nM) dramatically enhanced ROS production and induced a time-dependent increase in mRNA levels of inducible nitric oxide synthase (iNOS), NAPDH oxidase 2 subunit cybb, and FOS-like antigen 1 (fosl1), a marker for neuronal activation and a component of transcription factor activator protein-1 (AP-1). The maximum mRNA increase in these genes occurred 6 h following incubation (iNOS: 201-fold; cybb: 2 -fold; Ffosl1: 11-fold). The increases in iNOS and cybb mRNA were not attenuated by the AT₁ receptor antagonist losartan but abolished by the AP-1 blocker curcumin. Our results suggest that PVN PRR activation induces sympathoexcitation possibly through stimulation of an ANG II-independent, ROS-AP-1-iNOS signaling pathway



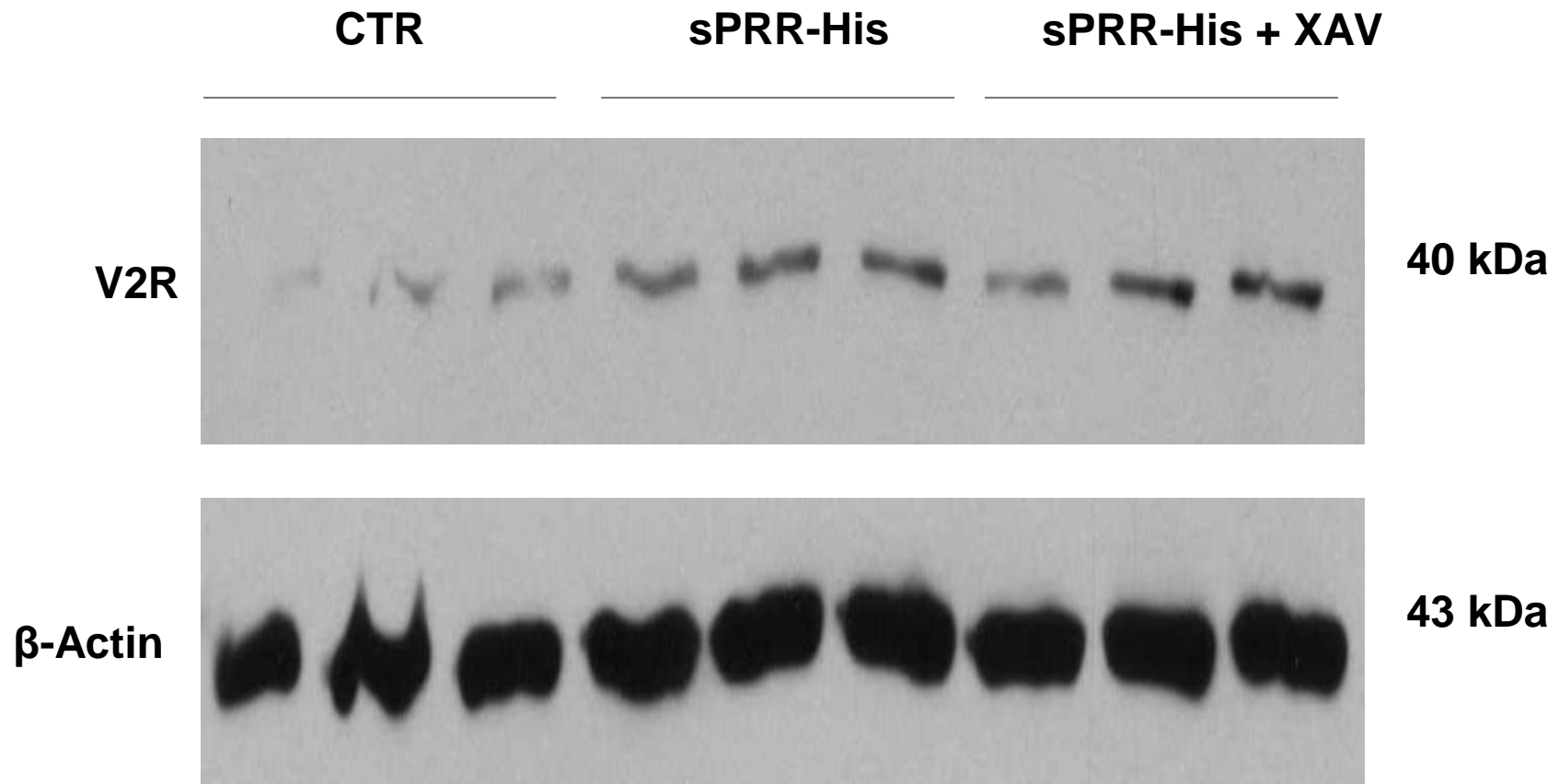
Central PRR

- Within the CNS, PRR is predominantly expressed in cardiovascular-relevant neurons including the paraventricular (PVN) and supraoptic nuclei (SON) and the subfornical organ (SFO) (Shan Z et al. 2010; Takahashi K et al. 2010)
- PRR is co-localized with vasopressin (AVP) and oxytocin in SON and PVN in humans (Takahashi K et al. 2010)
- PRR knockdown in the SON of spontaneously hypertensive rats (SHR) decreases MAP and heart rate and plasma AVP level (Shan Z et al. 2010)
- Conversely, in the same study, targeted overexpression of human PRR in the SON of Wistar-Kyoto (WKY) rats induced a 2-fold increase in plasma AVP and a 3-fold increase in urinary AVP, in parallel with enhancement of urine concentrating capability (Shan Z et al. 2010)
- Neuron-specific deletion of PRR or intracerebroventricular (ICV) infusion of a PRR decoy inhibitor PRO20 attenuates the hypertension induced by AngII or DOCA-salt (Li W et al. 2012; Li W et al. 2014; Li W et al. 2014).

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).



sPRR Stimulated V2R Expression in Cultured IMCD Cells



Immunostaining with Antibodies Recognizing Different PRR Domains

PRR (350 AA, 37-43 kDa)

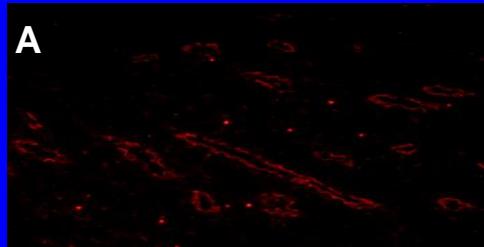


← Anti-PRR-N antibody

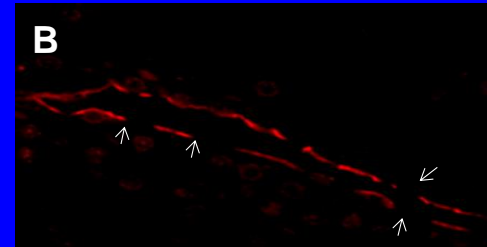
← Furin or ADAM19

← Anti-PRR-C antibody

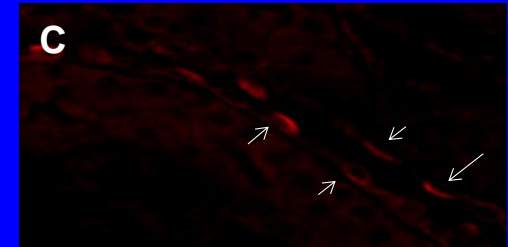
Anti-PRR-N antibody
200X



Anti-PRR-N antibody
400X



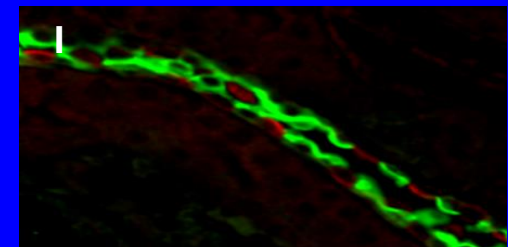
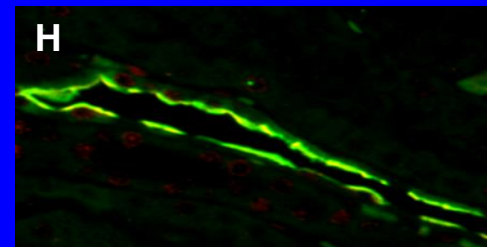
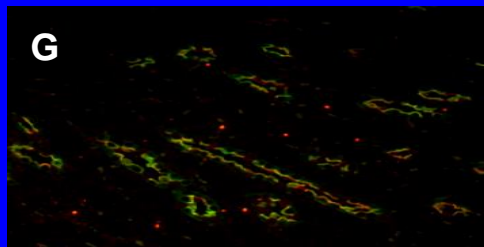
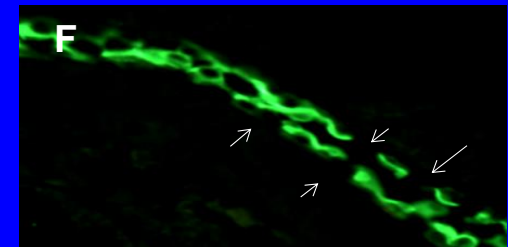
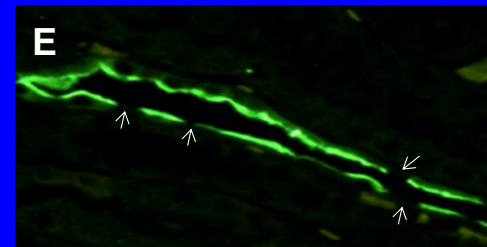
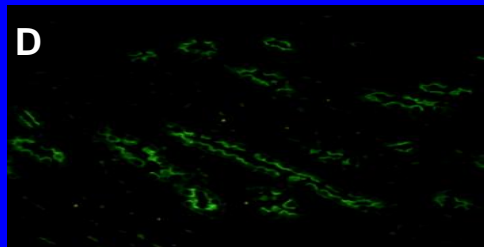
Anti-PRR-C antibody
400X



PRR

AQP2

Merge

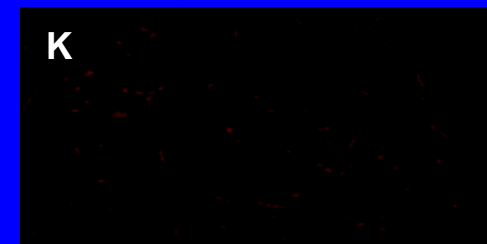
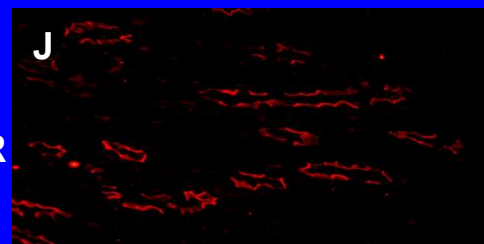


Anti-PRR-N antibody

Anti-PRR-N antibody + sPRR-His

Absence of primary antibody

PRR



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