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Angiotensin II increases the expression of (pro)renin receptor during low salt conditions in rat renal inner medullary collecting ducts

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Abstract

Introduction—Evidence indicates that chronic angiotensin II (AngII) infusion increases (pro)renin receptor ((P)RR) expression in rat renal inner medullary collecting ducts (IMCDs) cells. Recently, it was shown that renal (P)RR expression is augmented during low salt (LS) diet. However, the role of AngII in mediating the stimulation of (P)RR during LS conditions is unknown. We hypothesized that AngII mediates the increased expression of (P)RR during low salt conditions in IMCDs.

Methods—(P)RR expression and AngII levels were evaluated in Sprague Dawley rats fed a LS diet (0.03% NaCl) and normal salt (NS; 0.4% NaCl) during 7 days. In IMCDs isolated in hypertonic conditions (640 mOsm/L with 280 mM NaCl) we examined the effects of sodium reduction (130 mM NaCl) and AngII on (P)RR expression.

Results—Plasma renin activity in LS rats was significantly higher than rats fed with NS (28.1±2.2 vs. 6.7±1.1 ng AngI/mL/h; $P<0.05$), as well as renin content in renal cortex and medulla. (P)RR mRNA and protein levels were higher in medullary tissues from LS rats; but did not change in the cortex. Intrarenal AngII was augmented in LS rats compared to NS (cortex: 710±113 vs. 277±86 fmol/g; $P<0.05$; medulla: 2093±125 vs. 1426±126 fmol/g; $P<0.05$). In cultured IMCDs, the expression of (P)RR was increased in response to LS or AngII treatment and potentiated by both treatments (both at 640 mOsm/L). These data indicate that (P)RR is augmented in medullary collecting ducts in response to LS and that this effect is further enhanced by the increased intrarenal AngII content.

Keywords

Low salt diet; Intrarenal Renin-Angiotensin System; Collecting Duct

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Disclosures

The authors report no conflicts.

Introduction

The (pro)renin receptor ((P)RR) is a 350-amino acid protein with a single transmembrane domain, that binds renin or prorenin, increasing renin catalytic activity and fully activating prorenin. [1–3] (P)RR has been localized in glomerular mesangial cells, podocytes, and acid-secreting cells in the collecting duct. [4–7] Rodent models that overexpress (P)RR develop cardiovascular, renal diseases; [8–11] and increased aldosterone production and high blood pressure. [12] Cell surface binding of renin or prorenin to (P)RR not only increases renin enzymatic activity but also triggers extracellular signal-regulated kinase 1/2 (ERK1/2) mitogen-activated protein kinase (MAPK) signaling pathway. [1, 2] Activation of MAPK/ERK1/2 pathway upregulates cyclooxygenase-2 (COX-2) expression in cardiac tissues[13] and renal cortex. [9, 14] More recently, we reported that (P)RR activation by recombinant prorenin stimulates COX-2 expression independent of AngII in rat inner medullary cells,[15] and that (P)RR mRNA levels are increased in collecting duct cells of AngII infused rats. [16]

In Sprague-Dawley rats chronically infused with AngII, there is increased synthesis and secretion of prorenin by the principal cells in the collecting duct[17–19], which concur with the augmentation of Ang II levels in the kidney [20, 21] and urine [22], despite suppression of renin in the juxtaglomerular cells. Little is known regarding the mechanisms that regulate the expression of tubular RAS during LS conditions. Recently, Shao et al. demonstrated the effects of a low-salt (LS) diet on intratubular angiotensinogen and AngII levels. The authors found augmented intrarenal renin and AngII contents with no changes in intratubular angiotensinogen levels. [23] These findings indicate that collecting duct-derived renin is increased in renal medullary tissues during a LS diet and suggest that membrane bound (P)RR in the collecting duct cells may interact with prorenin and renin secreted by the collecting duct cells, thus contributing to the enhancement of local renin enzymatic activity.

Recently it was shown that (P)RR expression is augmented by LS diet via cyclic GMP-protein kinase G pathway in renal tissues and mouse inner medullary collecting ducts (IMCDs) isolated in isotonic conditions. [24,25] Although no changes might be expected in tissue osmolality during LS diet, we considered to test the effects of sodium reduction under natural hyperosmotic conditions of the kidney inner medulla and mimicking the effect of tubular sodium depletion in freshly isolated IMCDs. In the present study, we hypothesized that AngII mediates the increased expression of (P)RR during low salt conditions in IMCDs.

METHODS

Experimental animals and sample collections

The Tulane Institutional Animal Care and Use Committee approved all experimental protocols. Male Sprague-Dawley rats (150 to 175 g; Charles River Laboratories, Wilmington, MA) were cage-housed and maintained in a temperature-controlled room with 12-hours light/dark cycle, with free access to tap water. Rats (n=6) were fed with low salt diet (LS) (0.03% NaCl; Ralston Purina, St. Louis, MO) for 7 days and compared with control rats (n=6) fed with normal salt (NS; 0.4% NaCl; Ralston Purina, St. Louis, MO). At day 6 the rats were placed in metabolic cages for collection of urine. Rats were killed by conscious decapitation to collect trunk blood samples for determination of plasma sodium

concentrations, plasma renin activity (PRA) and plasma Ang II concentration as previously described. [20] Kidneys were dissected into renal medulla and cortex for measurements of Ang II, renin content, RNA and protein extractions as described previously. [17, 20]

Osmolality in renal cortex, medulla, and urine

Renal tissue osmolality was measured using a vapor pressure osmometer (Model 5520, Wescor, Logan, UT). Cortex and inner medullas (40 mg) were placed microfuge tube and snap frozen in liquid nitrogen. Tissues were minced and centrifuged at 14,000 rpm and the supernatant (10 microliters) were used to measure osmolality. For urine osmolality, samples were centrifugated at 14,000 rpm and 10 microliters were used. Measurements were made from filter discs absorbed with tissue fluid or urines. Controls measurements using tissue fluid absorbed onto a filter disk or using tissue slices resulted in identical results. Proper calibration of the instrument was verified by measurement of standards prior to the measurement.

Kidney renin content (KRC) and AngII content in renal cortex and medulla

Renin content was determined indirectly by measuring renin activity normalized by tissue weight. For renin content assessment, kidney cortex and inner medulla of each kidney was immersed in cold KRC homogenization buffer (2.6 mM EDTA, 3.4 mM hydroxyquinoline, 5 mM ammonium acetate, 200 μM PMSF, and 0.256 μM dimercaprol), minced, and quickly homogenized. The homogenates were centrifuged at 4,000 rpm at 4°C for 30 min and the supernatants were used to generate 1:1,000 dilutions that were spiked with 1 μM synthetic renin tetradecapeptide substrate (Sigma). The generated Angiotensin I in the samples was then assayed using the Diasorin RIA kit (DiaSorin) at pH 6.0 according to the manufacturer's instructions. Ang II was extracted from renal cortices and medullas and assayed as previously described. [22, 23]

(Pro)renin receptor ((P)RR) and renin immunoblotting analyses

(P)RR protein levels were examined in renal cortex and medullas and freshly isolated Inner Medullary Collecting Ducts (IMCDs) using a polyclonal rabbit anti-(P)RR that recognizes the intracellular segment and the ectodomain (ATP6AP2, 1:400 dilution; Cat ID HPA003156; Sigma-Aldrich, St. Louis, MO) at a dilution 1:100, overnight, followed by incubation with an anti-rabbit secondary antibody at a 1:30,000 dilution. Renin antibody (Santa Cruz Biotechnology; Santa Cruz, CA) was used at 1:200 dilutions. All analyses by Western blotting were performed using the Odyssey detection system (LI-COR Biosciences, Lincoln, NE). Densitometric analyses were performed by normalization against β-actin (Santa Cruz Biotechnology; Santa Cruz, CA) or against total protein (urine).

(P)RR and renin transcript levels in renal tissues and IMCDs

Quantitative real-time RT-PCR (qRT-PCR) was performed to evaluate rat renin and (P)RR mRNA expression in tissues and isolated IMCDs using the TaqMan PCR system. Total RNA was isolated using RNeasy Mini Kit (Qiagen). Total RNA (20 ng) was used to amplify (P)RR mRNA using the primers described previously. [16] Renin expression was assessed

using primers and probes previously described. [26] Data were normalized against β-actin mRNA and expressed as percentage of control.

Freshly isolated IMCDs

IMCDs were prepared as described before in hypertonic conditions. [26] In brief, after quick kidney excision, inner medullary tissues were extracted and minced under sterile conditions and digested in 10 ml of hypertonic digestion media (DMEM-Ham's F-12, 20 mg of collagenase B, 7 mg of hyaluronidase, supplemented with 80 mM urea, and 130 mM NaCl; 640 mOsm/L), and incubated at 37°C under continuous agitation for 90 min. After centrifugation, the pellet was washed in pre-warmed hypertonic culture media without enzymes [DMEM-Ham's F-12, 80 mM urea, 130 mM NaCl, 10 mM HEPES, 2 mM L-glutamine, penicillin-streptomycin (10,000 U/ml), 50 nM hydrocortisone, 5 pM 3,3,5-triiodothyronine, 1 nM sodium selenate, 5 mg/l transferrin, without FBS (pH 7.4, 640 mOsmol/kgH2O)]. The resulting IMCDs pellet was suspended in hypertonic media (640 mOsm/L, supplemented with 130 mmol/L NaCl; to reach 280 mEq/L NaCl and 80 mmol/L urea). After 6–8 hours of adapting period, cell culture media was replaced by fresh control hypertonic media (640 mOsm/L) or LS conditions (regular isotonic media supplemented with 10 mmol/L NaCl clamped at 640 mOsm/L with D-mannitol) and seeded in 3 mm petri-dishes and incubated with vehicle (PBS), AngII or AngII plus candesartan at 6 micromol/L (Sigma Chemical Co, St. Louis, MO) for 6–8 hours. A mixture of DMEM/F12 supplemented with 10 mmol/L NaCl. D-mannitol was used to clamp hyperosmotic conditions under LS conditions at 240 or 320 mmol/L.

Immunofluorescence in freshly isolated IMCDs

Methanol-fixed IMCDs were stained with a rabbit anti-aquaporin-2 (AQP2; Calbiochem, San Diego, CA) or a polyclonal rabbit anti-(P)RR (ATP6AP2, Cat ID HPA003156; Sigma-Aldrich, St. Louis, MO) at 1:200 dilutions. Secondary antibody (Alexa Fluor 488, Invitrogen, Carlsbad, CA) was used. Samples were counterstained with 4prime;,6-diamidino-2-phenylindole (DAPI, Invitrogen, Carlsbad CA) for nuclei staining. Negative controls were obtained by omission of the specific primary antibody.

Statistical Analysis

In vivo studies were performed using n=6 rats per group. Kidney renin content, tissue osmolality, kidney Ang II content and protein measurements were done in kidneys from 4 animals and in triplicates. For *in vitro* studies an average number of 6 to 8 independent observations were performed for each treatment. Data were evaluated by the Grubb test followed when appropriate by paired and unpaired Student t-test or by one-way ANOVA with Tukey post-test. For mRNA and protein data, control levels were defined as 100%. Significance was defined as $P<0.05$. Results are expressed as mean ± SEM.

RESULTS

Plasma Renin Activity (PRA), Plasma AngII levels, Urine and Tissue Osmolality

Table 1 shows the physiological parameters of the rats after 7 days feeding a NS or LS diet. PRA and plasma AngII levels were increased in LS animals. No changes in plasma sodium

concentrations were observed. Urinary sodium excretion and urinary osmolality were reduced in LS animals. No changes were observed in tissue osmolality between NS and LS diet in cortex and inner medullary tissues.

LS Diet Increases Intrarenal AngII and Kidney Renin Contents

As displayed in Table 1, LS diet increased intrarenal AngII content in renal cortex and medullary tissues. Kidney renin content (KRC) was significantly higher in cortical (LS: 1221 ± 141 vs. NS: 803 ± 71 ng AngI/hr/mg, $P<0.05$) and medullary tissues (LS: 5267 ± 1267 vs. NS: 2461 ± 672 ng AngI/hr/mg, $P<0.05$).

LS diet stimulates (P)RR expression in the kidney inner medulla

Figure 1A shows a representative mRNA levels in renal cortex and medullary tissues from rats fed a NS or LS diet (n=4). (P)RR mRNA was increased only in renal inner medulla (LS: 155 ± 21 % vs. NS: 100 ± 7 %; $P<0.05$) but not cortex. Figure 1B shows a representative Western blot of the (P)RR protein abundance. The (P)RR/β-actin ratio was increased in medullary tissues from rats fed with LS (150 ± 19 % vs. 100 ± 4 %; $P<0.05$). No changes were observed in renal cortex (LS: 106 ± 12 % vs. NS: 100 ± 11 %; $P<0.05$).

LS diet augments renin expression in cortex and the inner medulla

Figure 2A shows renin mRNA levels in kidney cortex and medullary tissues in NS and LS rats. LS treatment during 7 days increased renin synthesis in renal cortex (180 ± 40 % vs. 100 ± 27 %, $P<0.05$) as reflected by KRC values reported in Table 1. Renin synthesis in medullary tissues was also augmented (218 ± 57 % vs. 100 ± 19 %, $P<0.05$). Homogenates of cortical tissues and inner medullary tissues showed an increased renin protein levels (cortex: 178 ± 43 % vs. 100 ± 13 %, $P<0.05$, medulla: 213 ± 14 vs. 100 ± 25%, $P<0.05$, Figure 2B).

Full-length form of the (P)RR is augmented in urine samples of rats feed a LS diet

Urine collections (24 hrs) from NS and LS were 10X concentrated and subsequently subjected to Western blot analysis (40 micrograms of total protein) using a polyclonal rabbit anti-(P)RR that recognizes the intracellular segment and the ecto-domain (ATP6AP2, 1:200 dilution; Sigma-Aldrich) as described previously. [16] Membranes were incubated with secondary antibody RDye® Infrared Dye (LI-COR Biosciences, Lincoln, NE) and values normalized by urinary total protein. As observed in figure 3A, in rats fed with LS the full-length form of the (P)RR was significantly higher than NS (LS: 265 ± 80 % vs. NS: 100 ± 6 %; $P<0.05$, Figure 3B). Soluble form of (P)RR was not detected and may be not secreted under these conditions. Figure 3A also shows non-specific staining of major urinary proteins of low molecular weight using the same membrane as a non-quantitative loading control (~ 21 kDa).

AngII increases (P)RR expression in IMCDs in normal and LS conditions

Finally, we tested the effect of LS conditions in freshly isolated IMCDs grown in hyperosmotic media (640 mOsmol/L) to establish appropriate growth conditions with preferential selectivity for IMCD principal and intercalated cells. [27–29] Figure 4A shows

freshly isolated IMCDs stained with AQP-2 (a typical marker of principal cells of the collecting duct, green color) and (P)RR in apical membrane and the cytoplasm of intercalated cells (green and arrows), confirming previous observations. [15] We tested the effect of AngII in IMCDs maintained hypertonic media (640 mOsmol/L, supplemented with 130 mM NaCl and 80 mM urea, total ~280 mM NaCl). The (P)RR mRNA levels were significantly augmented by AngII (mRNA: 125 ± 7 % vs. 100 ± 3 %; P<0.05). Protein levels were also augmented by AngII treatment (146 ± 10 % vs. 100 ± 2 %; P<0.05). Candesartan, an AT1 receptor blocker, suppressed this effect (Figure 4B). LS conditions (regular media supplemented with 10 mMol/L NaCl, 80 mM urea and clamped with 240 mMol/L mannitol) caused a significant increase in (P)RR expression as compared to controls (mRNA: 145 ± 4 %; protein: 161 ± 14 %; P<0.05) this effect was enhanced by AngII treatment (mRNA: 158 ± 5 %; protein: 193 ± 3 %; P<0.001 versus controls). To test the effect of the absence of urea in the media we performed another experiment to evaluate LS conditions in which urea was replaced by D-mannitol, clamping the osmolality to 640 mOsm/L. Interestingly, as shown in Figures 5A and B, LS conditions in the absence of urea caused a slight but non significant increase in (P)RR mRNA expression (115 ± 7 % versus 100 ± 3 %; P=0.14); as well as in (P)RR protein levels (116 ± 10 % versus 100 ± 2 %; P=0.18). However, AngII elicited a significant increase in (P)RR expression under this condition (mRNA: 179 ± 15 %, protein: 249 ± 17 %, P<0.001 versus controls).

DISCUSSION

The present study demonstrates that (P)RR mRNA and protein levels are increased in the renal inner medulla of Sprague-Dawley rats subjected to LS diet for 7 days. Tissue AngII levels in renal cortex and medulla from rats fed LS were markedly higher than rats fed a NS diet, furthermore, medullary AngII was significantly higher than renal cortex. In freshly isolated IMCDs cultured in hypertonic conditions, AngII treatment (100 nM, 6 hrs) increased (P)RR protein levels and AT1 receptor blockade suppressed this effect. Reduction in NaCl in culture media increased (P)RR despite the clamped hypertonicity. Importantly, AngII treatment exacerbate the stimulatory effect of LS on (P)RR expression *in vitro*.

Augmentation of (P)RR in the renal medulla has been previously observed in rats chronically infused with AngII.[16, 30] The same experimental model exhibits augmented renin and prorenin synthesis and secretion by the collecting duct cells. [22, 26] These observations are important in the light of recent evidence showing the binding capability of intratubular prorenin and renin by the (P)RR located on the apical plasma membrane of intercalated cells in collecting duct cells. [16] (P)RR in the collecting duct may further increase renin activity and fully activate prorenin, thus contributing to the intrarenal RAS activation during LS conditions. We previously showed that (P)RR activation increases COX-2 expression in rat renal medullary intercalated cells [15], which in turn might modulate the effects of AngII by enhancing prostaglandin synthesis [31] as previously proposed [15]. The activation of (P)RR has been also implicated in the development of kidney disease and inflammation, due to its ability to activate intracellular phosphorylation of MAPK/ERK1/2. [2, 7] Recent studies have shown that increased intrarenal RAS activity is not sufficient to cause renal injury as observed with chronic AngII infusions. [23] Indeed, during physiological intrarenal RAS activation due to a chronic LS diet in rats there is not a major

renal tissue injury response; even in the presence of high intrarenal Ang II. [23] Nonetheless, in the study by Shao et al., the only tissue injury response that resulted augmented was renal fibrosis, which might be evoked by the activation of (P)RR. [23, 24] Furthermore, the PRR-dependent stimulation of COX-2 in mesangial cells, macula densa, [14, 32] and the renal inner medulla [15] points out the need of further studies to examine the potential physiological roles of (P)RR in regulating sodium balance and blood pressure to buffer the excessive effects of AngII during intrarenal RAS activation. Our findings, along with the previous demonstration showing that rats fed a LS diet during 14 days there is increased renal renin content particularly in the renal medulla [23], suggest that chronic consumption of an extreme sodium restriction may stimulate the intrarenal RAS. [23, 24]

Matavelli et al, reported that (P)RR expression was augmented in response to LS diet in glomeruli, proximal, distal tubules and collecting ducts [24]. The authors also demonstrated that in rats fed a LS diet, (P)RR mRNA and protein decreased significantly in response to L-NAME and PKGi, suggesting that LS intake enhanced the renal expression of (P)RR via cGMP-PKG signaling pathway. [24] Similarly, the same group showed that LS exposure upregulates (P)RR expression via that cGMP-PKG/cAMP/CRE/nuclear factor-κB p65, and c-Jun in a mouse IMCD cell line grown in isotonic conditions. [25]

Although tissue osmolality was unaffected by LS diet in our study, urine osmolality, as well as sodium excretion were lower than NS rats. Coincidently with augmented (P)RR expression in renal medulla from rats fed a LS diet, renin activity in inner medulla, as well as mRNA and protein expression in IMCDs were increased as compared to NS rats. We have also shown that freshly isolated rat IMCDs grown in natural hyperosmotic conditions with preferential selectivity for IMCD principal and intercalated cells, [27–29] exhibited increased (P)RR mRNA levels after sodium reduction (from ~280 mmol/L NaCl to ~140 mmol/L).

It is known that renal inner medulla is subjected to extreme osmotic conditions, which are necessary for the function of the urinary concentrating mechanism. At present, several cell lines have either lost segment-specific characteristics, or the segment of origin is unknown. For that reason, we used freshly isolated IMCDs which retained many of the differentiated properties of this nephron segment, and importantly, the functional expression of renin (P)RR and AT1 receptors. [26] Because cortical and medullary osmolality were higher than the regular isosmotic conditions (~300 mosm/L), we cultured IMCDs cells under hyperosmotic condition as described previously for this kind of cell culture. [27–29] The fact that (P)RR gene expression in the renal cortical regions was not upregulated in the present study may suggest that natural hyperosmotic conditions in the renal inner medulla could exert different regulatory mechanisms among the various epithelial cells in the kidney. It is known that phenotypes in cortical and medullary collecting ducts are not identical, especially if we consider intercalated cell phenotype. [33] Even more, some of the transcription factor involved in the regulation of (P)RR under LS conditions can be also found to be active by changes in osmolality, [34, 35]. In the present study, although AngII increased (P)RR mRNA and protein levels in cultured IMCDs, a reduction in sodium concentration (near to half of normal hypertonic media) was able to produce a similar

increase (Figure 5). Interestingly, (P)RR upregulation by LS was even further potentiated by and AngII treatment, candesartan completely blocked this effect.

The (P)RR can be processed intracellularly by cleavage leading to a soluble form (s(P)RR) that is secreted into the plasma [36] and urine [16], being available to bind prorenin and renin enhancing its activity which might stimulate intrarenal AngII formation. Previous findings suggested that the s(P)RR in the renal medulla is stimulated in Ang II-dependent hypertension via furin-mediated cleavage of the full-length form. [16, 36] In this work, despite increased expression of the full-length form of the (P)RR in renal medullary tissues during LS diet, we were unable to detect the soluble form (s(P)RR), neither in renal tissues or in urines.

CONCLUSION

In summary, we demonstrated that LS diet increases intrarenal AngII levels and (P)RR expression in rat renal IMCDs. *In vitro* studies using freshly isolated IMCDs demonstrated that a reduction in sodium concentration in the cell media augments (P)RR levels and that AngII further increase this effect via an AT1R-mediated mechanism. Taking together, these findings along the concomitant stimulation of collecting duct renin and intrarenal AngII contents, suggest that (P)RR may contributes to the activation of intrarenal RAS during LS conditions. Although there is consensus that a moderate reduction in salt intake can help to decrease blood pressure in both hypertensive and non-hypertensive subjects [37], an extreme sodium diet restriction may lead to a marked activation of the intrarenal RAS, including the stimulation of (P)RR expression. Therefore, a moderate reduction in salt consumption should be considered to maintain blood pressure in optimal levels. [38, 39]

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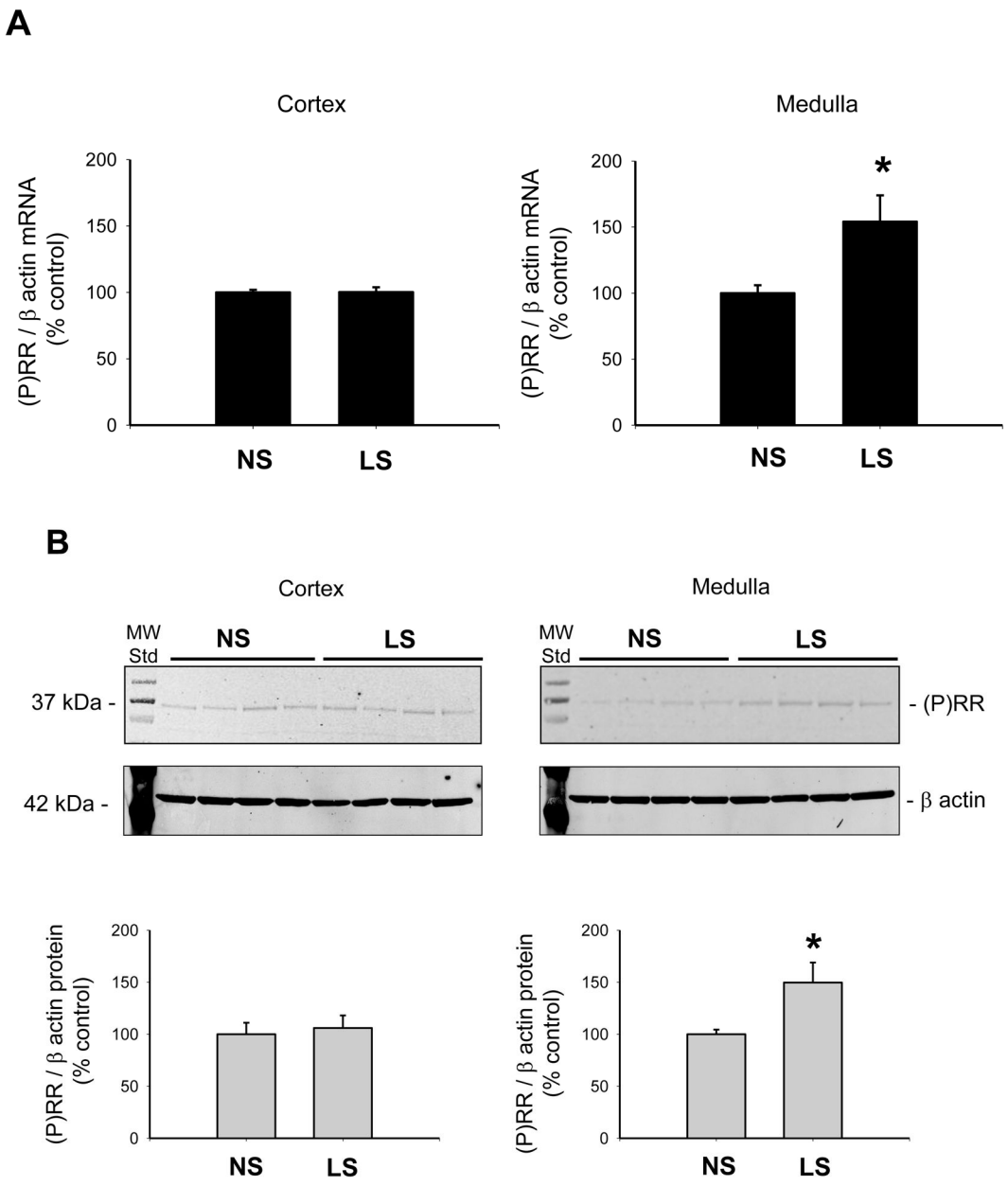

FIGURE 1.

Expression of the (P)RR in renal cortex and medulla of Sprague-Dawley rats after 7 days of dietary salt. **A.** Quantification of cortical and medullary (P)RR expression by qRT-PCR in LS and NS rats (n=6, * P<0.05). **B.** Representative immunoblots show (P)RR protein abundances in cortical and medullary tissues from four NS- and LS-animals. Data was normalized against β actin protein or mRNA levels. (n=4, * P<0.05).

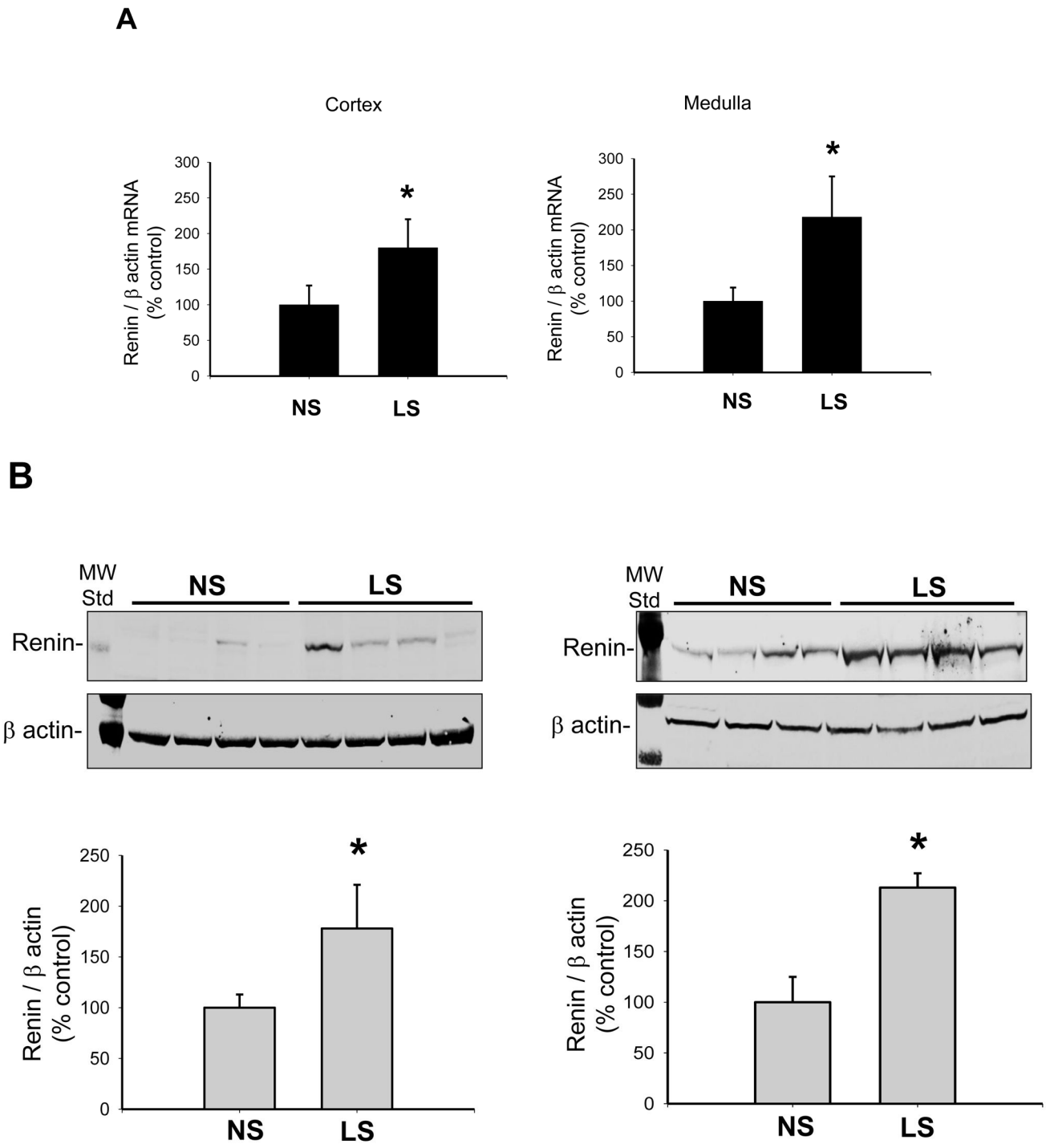

FIGURE 2.

Renin expression in renal cortex and medulla of Sprague-Dawley rats after 7 days of low sodium diet. **A.** Quantification of cortical and medullary renin mRNA expression by qRT-PCR in LS and NS rats (n=6, * P<0.05). **B.** Representative immunoblots show renin protein levels in medullary tissues from four NS and LS animals. Data was normalized against β actin protein levels (n=4, * P<0.05).

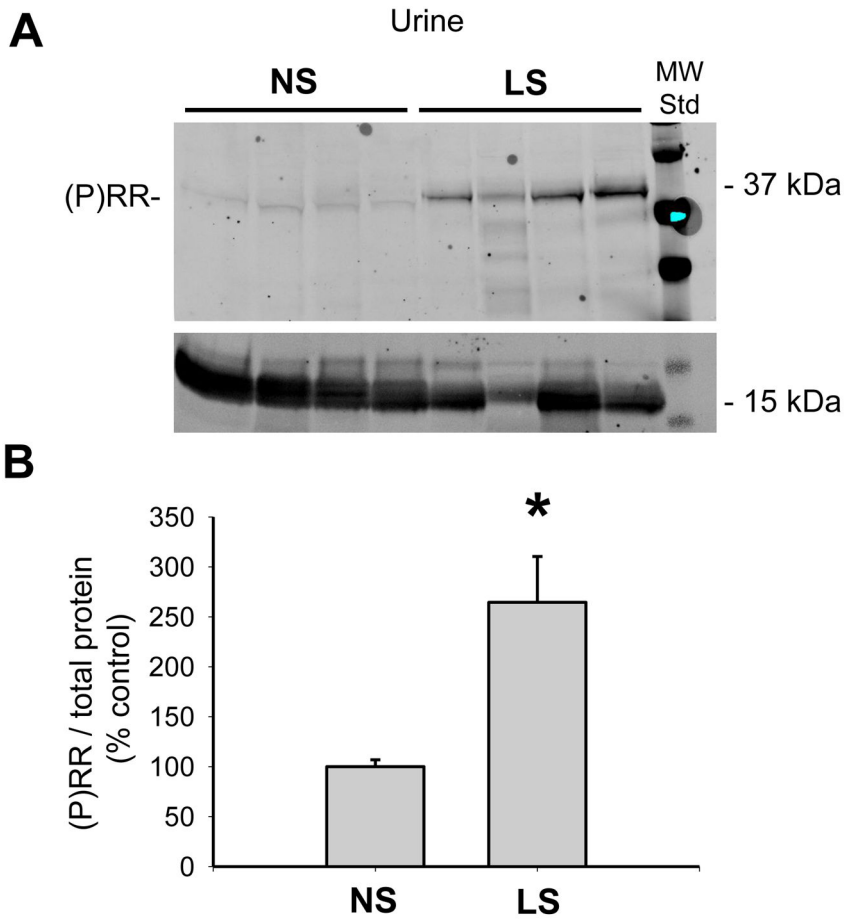

FIGURE 3.

(P)RR in urine samples from NS and LS Sprague-Dawley rats. **A.** 24 hour-urine collections were 10X concentrated and 40 micrograms loaded in each well (n=4). Samples were incubated with specific (P)RR antibody. As loading control, unspecific staining of major urinary proteins is also shown in the same membrane. **B.** Quantification of specific (P)RR immunoblots normalized by total protein (as percentage of NS rats, * $P<0.05$).

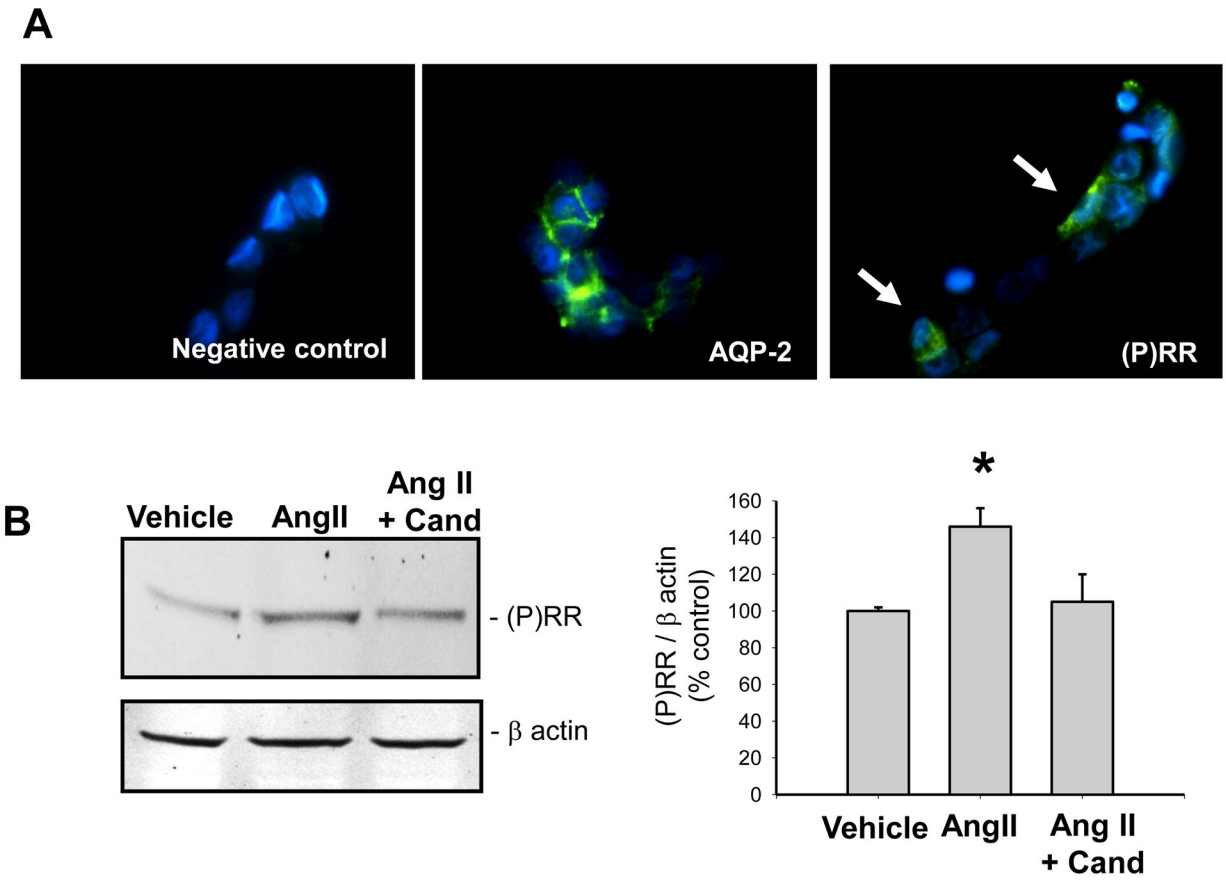

FIGURE 4.

A. Immunofluorescence images of rat IMCDs isolated in natural hyperosmotic conditions showing the expression of AQP-2, a typical marker of collecting duct principal cells and (P)RR in intercalated cells (arrows). Samples were counterstained with 4prime;,6-diamidino-2-phenylindole (DAPI) for nuclei staining. Negative controls were obtained by omission of the specific primary antibody. **B.** Western blot analysis and quantification of (P)RR expression in isolated rat IMCDs in response to AngII treatment and AngII plus candesartan (cand), an AT1 receptor blocker (n=6, * P<0.05).

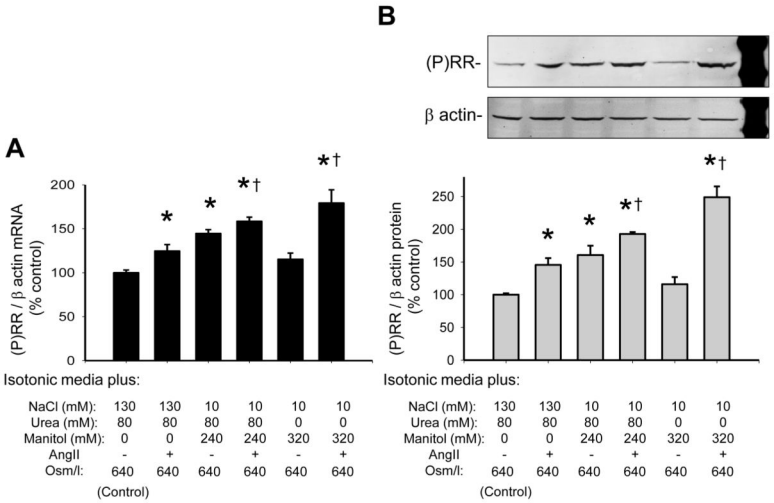

FIGURE 5.

Quantification of (P)RR mRNA and protein levels in response to low salt (LS) conditions and AngII (10^{-7} M) treatment in freshly isolated IMCDs isolated in hyperosmotic conditions. **A.** The mRNA levels were increased by LS and AngII, combined treatment enhanced this effect. **B.** A similar response was observed by analyzing (P)RR protein levels (n=6, *P<0.05 and †P<0.001 versus control cells isolated in hypertonic media by adding 130 mM NaCl plus 80 mM Urea).

TABLE 1

Physiological parameters of Sprague-Dawley rats subjected to a low and normal salt diet for 7 days

	NS	LS
Plasma renin activity (ng Ang I/mL/h)	6.7 ± 1.1	28.1 ± 2.2 *
Plasma AngII (fmol/mL)	28.8 ± 4.7	116.0 ± 14.6 *
Plasma Na^+ (mEq/L)	140 ± 4	138 ± 3
Urinary Na^+ excretion (mEq/24h)	162 ± 6	0.7 ± 0.2 *
Urine osmolality (mosmol/Kg H_2O)	1343 ± 87	686 ± 101 *
Tissue osmolality (mosmol/Kg H_2O)		
Cortex	641 ± 42	703 ± 70
Medulla	1250 ± 300	1374 ± 150
Intrarenal AngII content (fmol/g)		
Cortex	277 ± 86	710 ± 113 *
Medulla	1426 ± 126	2093 ± 125 *
Kidney renin content (ng Ang I/hr/mg)		
Cortex	803 ± 71	1221 ± 141 *
Medulla	2461 ± 672	5267 ± 1267 *