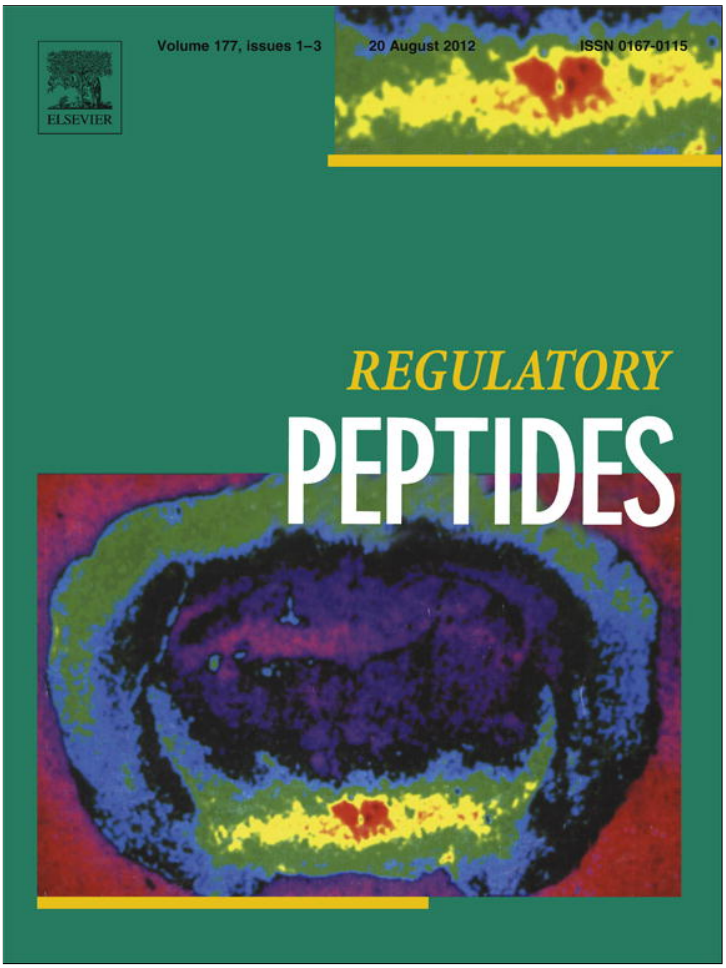
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Salt-induced downregulation of renal aquaporins is prevented by losartan

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ABSTRACT

Aims: The purpose of this study was to investigate the expression of aquaporin-1 (AQP-1) and aquaporin-2 (AQP-2) in the renal tubule of rats fed with a high-salt diet and its modulation by the AT1 receptor blocker losartan.

Main methods: The experiments were performed in four groups of rats fed for 3 weeks with the following diets: regular rat chow (NS); high-salt (8% NaCl) chow (HS), NS plus losartan (NS-L) and HS plus losartan (HS-L). Losartan (40 $mg.kg^{-1}$) was administered in the drinking water. Systolic blood pressure (SBP) and renal function were evaluated. The intrarenal levels of angiotensin II (Ang II), TGF-β_1, α-smooth muscle actin (α-SMA), endothelial nitric oxide synthase (eNOS), AQP-1 and AQP-2 were determined by immunohistochemistry. AQP-1 and AQP-2 protein levels were measured by western blot analysis.

Key findings: A high-sodium diet downregulated AQP-1 and AQP-2 expression levels in the proximal tubule and collecting duct, respectively. The high-sodium diet also induced Ang II, TGF-β_1 and α-SMA overexpression and decreased eNOS expression in the renal cortex and medulla. Losartan increased the diuresis and natriuresis, favoring urinary sodium concentration. Additionally, losartan prevented the profibrogenic response, decreasing Ang II, TGF-β_1 and α-SMA levels and normalizing AQP-2 expression in the HS-L group. AQP-1 expression was upregulated by losartan in both the NS-L and HS-L groups.

Significance: These results show that increased intrarenal Ang II in rats fed with a high-salt diet downregulates renal AQP-1 and AQP-2 expressions. In addition, although losartan increased diuresis and natriuresis, it prevented the downregulation of aquaporins, favoring urinary sodium concentration.

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1. Introduction

Diverse factors like AQP-1, AQP-2, Ang II and vasopressin can alter the ability of the kidney to recover water from the tubular fluid. AQP-1 mediates the reabsorption of already 80% of the fluid from the glomerular filtrate, being expressed in the luminal and basolateral membranes of the proximal tubules, in epithelial cells of the thin descending limb of Henle's loop and in endothelial cells of the descending vasa recta [1,2]. In addition, AQP-2 channels reabsorb the remaining 20% of the tubular fluid in the collecting duct. Vasopressin, besides its known properties to stimulate water reabsorption, translocates AQP-2 from the intracellular vesicles to the apical plasma membrane in renal tubular cells and stimulates AQP-2 gene transcription [3].

The renin–angiotensin system is a major hormonal regulator of sodium homeostasis and mediates most of its effects via Ang II [4]. Ang II signals are mediated by stimulation of two receptor subtypes, named Ang II type 1 (AT1) and Ang II type 2 (AT2). AT1 receptors are ubiquitously expressed in the kidney, mediate vasoconstriction of the afferent and efferent arterioles and medullary microvasculature and modulate the glomerular filtration rate, the tubuloglomerular feedback mechanism, and cell growth. In addition, Ang II modulates also the activity of diverse ion transporters in the kidney, such as Na^+/H^+ interchanger, NKCC2 co-transporter, NCC co-transporter and ENaCs [5]. Altogether, these actions of Ang II contribute in a synergistic manner to increase the capability of the kidneys to conserve sodium and maintain blood pressure within normal levels. AT2 receptors are localized in glomerular epithelial cells, proximal tubules, collecting ducts and in renal vasculature. They are considered as functional antagonists of AT1 receptors, and are associated with vasodilatation, apoptosis, antiproliferation and the increase of natriuresis, by stimulating the NO/cGMP/protein kinase G pathway [6]. It has been recently demonstrated that Ang II regulates also the activity of the main AQPs in the kidney. Wintour et al. reported that the in vivo infusion of Ang II increased AQP-1 gene expression in the sheep kidney [7]. Moreover, it has been demonstrated that Ang II may regulate AQP-2 targeting to the plasma membrane in the inner medullar collecting duct cells, through AT1

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receptor activation [8], and that the peptide also potentiates vasopressin effects on AQP-2 [9]. In addition, Bouley et al. [10] have shown a biphasic effect of exogenous Ang II administration, since 10^{-8} M to 10^{-9} M Ang II upregulated and a higher concentration of Ang II (10^{-7} M) downregulated AQP-1 mRNA expression in proximal tubule cells. Furthermore, it was also documented that an autonomous renin–angiotensin system is present in renal proximal tubules, being Ang II locally produced and secreted, reaching a concentration that is 100-fold higher in the lumen than in plasma [11]. The facts that renin and its mRNA expression were localized in the principal cells of the collecting ducts [12], that angiotensin converting enzyme (ACE) was found in distal nephron segments and that angiotensinogen was found in the urine [13], indicates that the distal part of the nephron is also a place of synthesis of intrarenal Ang II. In this way, locally formed Ang II in the kidney, in pathological conditions, may contribute to renal diseases by inducing sodium retention, inflammation, fibrosis [14] and AQP downregulation [15,16].

Up to date, there are no reports analyzing the effects of a chronic overload of salt on AQP expression in the kidney. Rats fed with a high-sodium diet constitute an experimental model, characterized by low plasma renin activity and circulating Ang II levels but with increased Ang II intrarenal levels [17], which is useful to analyze that relationship. Based on these antecedents, we hypothesized that the increase of renal Ang II observed in rats subjected to sodium overload could downregulate AQP expression through a proinflammatory and profibrotic effect, decreasing urinary sodium concentration. Then, the aim of the present study was to examine the immunoexpression of AQP-1 and AQP-2 in rats subjected to a high-salt diet, with or without simultaneous Ang II-AT1 receptor blockade.

2. Materials and methods

2.1. Animal protocol

Male Sprague Dawley rats of 200–230 g body weight were used. Animals were housed in steel cages in a controlled temperature room at 23 ± 2 °C, exposed to a daily 12-hour light–dark cycle (lights on at 07:00 a.m. and off at 07:00 p.m.), and fed for 3 weeks with the diets described below, with free access to tap water. Experiments were conducted in accordance with the institutional guidelines for the care and use of research animals of the University of Buenos Aires, Argentina.

Animals were randomly divided into four groups (n = 6 each group): a) NS (control): animals fed with a normosodic diet (0.4 g% NaCl); b) HS: animals fed with a high-sodium diet (8 g% NaCl); c) NS-L: animals fed with a normosodic diet plus losartan (40 $mg.kg^{-1}.day^{-1}$; Sigma-Aldrich Inc., St. Louis, Missouri, USA) administered together with the drinking water; d) HS-L: animals fed with a high-sodium diet plus losartan. At the end of the third week, the systolic blood pressure (SBP) was measured by the tail cuff method and recorded in a Grass Polygraph 79D, before the sacrifice of the animals by decapitation. The kidneys were quickly removed and processed for histological studies which include the measurement of intrarenal levels of Ang II, TGF-β_1, α-smooth muscle actin (α-SMA) and endothelial nitric oxide synthase (eNOS) by immunohistochemistry and AQP-1 and AQP-2 by western blot analysis.

To evaluate the renal function, additional four groups were performed. The last day of the third week, the animals were intraperitoneally anesthetized with urethane (1.2 $g.kg^{-1}$). A PE-90 tube (3 cm long) was inserted into the trachea to maintain an open airway and the left femoral vein was catheterized with a Silastic cannula (0.12 mm i.d.) for continuous infusion. The bladder was cannulated for urine collection using a PE-75 cannula. The femoral vein was infused with 0.15 M NaCl isotonic saline solution (ISS) at a rate of 0.04 $mL.min^{-1}$ (Syringe Infusion Pump, Sage™, Orion) for 60 min, to achieve a steady diuresis and allow urine collection (equilibration period). Then, the ISS infusion continued for another 60 min at the same rate (experimental period). A blood sample was collected after 30 min of the experimental period and a urine sample was collected during 60 min of the experimental period, for measurement of urine volume, urinary and plasma sodium and creatinine.

2.2. Urine and blood measurements

Urinary and plasma sodium and creatinine were measured by standard methods using an autoanalyzer. Creatinine clearance was assessed to evaluate the glomerular filtration rate (GFR). GFR and sodium fractional excretion (FE_{Na}) were calculated according to a standard formula. Urinary flow (UV) is expressed as $\mu L.min^{-1}.kg^{-1}$, sodium urinary excretion (UV_{Na}) as $\mu mol.min^{-1}.kg^{-1}$, urinary sodium concentration (U_{Na}) as $mEq.L^{-1}$, GFR as $mL.min^{-1}$ and FE_{Na} as percentage.

2.3. Kidney processing for histological studies

At the end of the experimental period, both kidneys were rapidly excised, decapsuled, longitudinally cut, fixed in formol-buffer (pH 7.2) and then included in paraffin and cut into 3-μm sections. For immunohistochemistry, sections were deparaffined and rehydrated, and endogenous peroxidase activity was blocked by treatment with 0.5% H_2O_2 in methanol for 20 min. Local expression of AQP-1, AQP-2, Ang II, TGF-β_1, α-SMA and eNOS was detected using the following specific antibodies: AQP-1 (Santa Cruz Biotechnology, Inc., USA; 1:50 dilution), AQP-2 (Santa Cruz Biotechnology, Inc.; 1:200 dilution), human anti-Ang II (Península, CA; 1:500 dilution), rabbit anti-TGF-β_1 (Santa Cruz Biotechnology, Inc.; 1:200 dilution), rabbit anti-α-SMA (Santa Cruz Biotechnology, Inc.; dilution of 1:200), and rabbit anti-eNOS (Santa Cruz Biotechnology, Inc.; 1:200 dilution). Immunostaining was carried out by means of a commercially modified avidin–biotin–peroxidase complex technique (Vectastain ABC kit, Universal Elite, Vector Laboratories, CA, USA) and counterstained with hematoxylin. Histological sections were observed in a Nikon E400 light microscope (Nikon Instrument Group, Melville, New York, USA). All measurements were carried out using an image analysis software (Image-Pro Plus ver. 4.5 for Windows, Media Cybernetics, LP, Silver Spring, MD, USA). The results are expressed as percentage of the stained area.

2.4. Kidney processing for western blot analysis of aquaporins

Kidneys were excised and the renal cortex and medulla were immediately dissected. Tissue samples were homogenized on ice with a Tissue Tearor (Biospec Products Inc.) in a buffer mixture (50 $mmol.L^{-1}$ Tris, 0.1 $mmol.L^{-1}$ EDTA, 0.1 $mmol.L^{-1}$ EGTA, 1% Triton, 1 $mmol.L^{-1}$ PMSF, 1 $\mu mol.L^{-1}$ pepstatin, 2 $\mu mol.L^{-1}$ leupeptin, 1× protease inhibitor cocktail (Roche Diagnostics)). Protein concentration was determined by Lowry technique in the Triton-soluble supernatant. Samples of cortex and inner medulla containing similar amounts of protein (100 μg protein/lane) were separated by electrophoresis in 7.5% SDS-polyacrylamide gels (Bio-Rad, Munich, Germany), transferred to a nitrocellulose membrane (Bio-Rad), and then incubated with rabbit polyclonal anti-AQP-1 (Santa Cruz Biotechnology, 1:250 dilution), or rabbit polyclonal anti-AQP-2 (Santa Cruz Biotechnology,1:250 dilution). A secondary immunoreaction with a goat anti-rabbit antibody conjugated with horseradish peroxidase (1:5000 dilution) was then carried out. The samples were revealed by chemiluminescence using ECL reagent for 2–4 min (Amersham Pharmacia Biotech). The density of the respective bands was quantified by densitometric scanning of Western blots using a Hewlett–Packard scanner and a Totallab analyzer software (Biodynamics Corp., Seattle, WA). Protein amounts were calculated by densitometry. Protein levels are expressed as the ratio between AQP1 or AQP-2 and β-actin bands (Assay Designs Inc) and control optical densities.

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2.5. Statistical analysis

Results of urine and blood measurements and SBP are expressed as mean ± SEM (standard error of the mean). Immunostains are expressed as the percentage of the positively stained area ± SEM. The Gaussian distribution was evaluated by the Kolmogorov and Smirnov method. The groups were compared using ANOVA followed by the Newman–Keuls test. p values <0.05 were considered significant.

3. Results

3.1. Systolic arterial pressure and renal function

The SBP increased in the HS group with respect to the NS group (mm Hg, NS: 128 ± 2, HS: 148 ± 2, $p<0.01$; ΔSBP: 20 ± 2). Losartan reduced the SBP in the NS-L and HS-L groups, but with a difference in tensional levels that is very similar to that observed between the NS and HS groups (NS-L: 107 ± 2, HS-L: 122 ± 4, $p<0.05$; ΔSBP: 15 ± 2).

Table 1 shows the parameters of renal function. UV increased in the HS group as compared with the NS group. Losartan did not alter UV in the NS-L group, but increased UV further in the HS-L group. UV_{Na} exhibited a behavior very similar to UV: it was higher in the HS group than in the NS group. Losartan did not modify UV_{Na} in the NS-L group, but increased it further in the HS-L group. U_{Na} increased in the HS group as compared with the NS group; whereas losartan increased U_{Na} in both the NS-L and the HS-L groups, maintaining a significant difference between the NS-L and HS-L groups. GFR was unaltered in the HS group with respect to the NS group. Losartan diminished GFR in the NS-L group, but did not alter it in the HS-L group. FE_{Na} increased in HS with respect to NS; whereas losartan increased FE_{Na} in both the NS-L and the HS-L groups, but with a difference between their FE_{Na} values similar to that observed between the NS and HS groups.

3.2. Expression of aquaporins in renal sections

Fig. 1 shows AQP-1 and AQP-2 abundance determined by western blot. AQP-1 expression was slightly but significantly diminished in the HS group with respect to the NS group. Losartan administration increased AQP-1 in both the NS-L and HS-L groups. AQP-2 was significantly decreased in the HS group compared with NS rats and was normalized to control levels by losartan (HS-L group).

Fig. 2 shows the immunostaining of AQP-1 in renal tissue. AQP-1 immunoexpression was decreased in proximal tubules of HS animals, and increased by the administration of losartan in both the NS-L and HS-L groups.

Fig. 3 shows the immunostaining of AQP-2 in renal tissue. AQP-2 immunoexpression decreased in the cortical and medullar collecting ducts of the HS group. This decrease was prevented by losartan administration.

Table 1
Renal function parameters.

	UV	UV_{Na}	U_{Na}	GFR	FE_{Na}
NS	10.1 ± 2.2	0.4 ± 0.3	19.6 ± 5.2	1.5 ± 0.4	0.06 ± 0.05
HS	16.1 ± 2.1*	2.9 ± 1.6*	164.7 ± 16.7*	1.3 ± 0.3	0.51 ± 0.24*
NS-L	8.6 ± 2.1	0.5 ± 0.1	59.3 ± 9.1†	0.4 ± 0.1†	0.25 ± 0.09†
HS-L	27 ± 1.4*†	8.5 ± 0.5*†	315.0 ± 23.5*†	1.5 ± 0.5*	1.3 ± 0.01*

UV: urinary flow ($\mu L.min^{-1}.kg^{-1}$); UV_{Na}: urinary sodium excretion ($\mu Eq.min^{-1}.kg^{-1}$); U_{Na}: urinary sodium concentration ($mEq.L^{-1}$), GFR: glomerular filtration rate ($mL.min^{-1}$); FE_{Na}: sodium fractional excretion (%). NS: normosodic diet; HS: high-sodium diet; NS-L: normosodic diet plus losartan; HS-L: high-sodium diet plus losartan. Values are expressed as mean ± SEM. *$p<0.05$ vs. the respective normosodic control (NS and NS-L); †$p<0.01$ vs. the respective control without losartan (NS and HS).

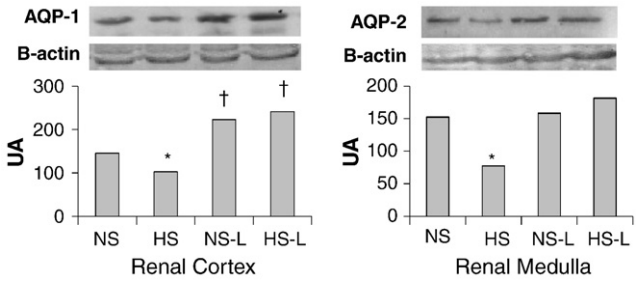


Fig. 1. Western blot of AQP-1 and AQP-2. Representative western blot analysis of AQP-1 abundance in the renal cortex and AQP-2 abundance in the renal medulla in the following groups: NS: normosodic diet; HS: high-sodium diet; NS-L: normosodic diet plus losartan; HS-L: high-sodium diet plus losartan. Values [means ± SEM (n = 5)] represent the mean of densitometric units (DU) after background subtraction. All experiments were performed in triplicate. Each blot was normalized to ß-actin expression band in the same gel. *$p<0.01$ vs. the respective control without losartan (NS and HS).

3.3. Immunohistochemical staining of Ang II and profibrotic markers in renal sections

Table 2 and Figs. 4 to 7 show the immunostaining of Ang II, TGF-β_1, α-SMA and eNOS in renal tissues, respectively. Immunoexpression of Ang II (Table 2 and Fig. 4) and TGF-β_1 (Table 2 and Fig. 5) increased in renal tubular cells of the HS group, while that of α-SMA (Table 2 and Fig. 6) increased in the interstitium of the renal cortex and medulla. Losartan (HS-L group) prevented the increase in Ang II, TGF-β_1 and α-SMA expressions elicited by the sodium load, in the renal cortex and the medulla. On the other hand, losartan treatment did not alter Ang II and TGF-β_1 staining in the NS-L group, although it slightly increased α-SMA staining in the renal cortex and the medulla in the mentioned group.

Conversely, the intrarenal expression of eNOS (Table 2 and Fig. 7) was markedly decreased in tubular cells of the renal cortex and the

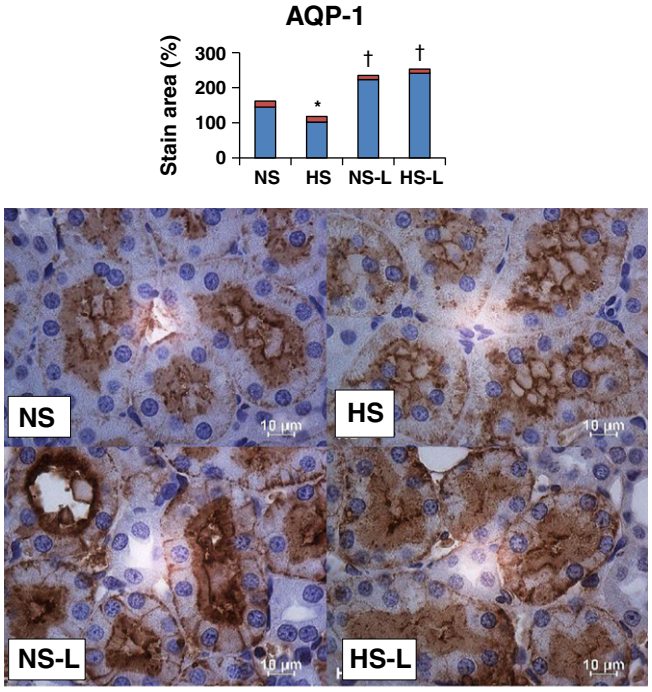


Fig. 2. AQP-1 immunoexpression in renal tissues. Assessment of the immunohistochemical expression of AQP-1 in renal tissue. NS: normosodic diet; HS: high-sodium diet; NS-L: normosodic diet plus losartan; HS-L: high-sodium diet plus losartan. Values are expressed as percentage (%) of the positively stained area ± SEM; n = 5; *$p<0.01$ vs the NS group; †$p<0.01$ vs the respective control without losartan. Representative image of positive staining corresponding to AQP-2 expression in the renal cortex (400×).

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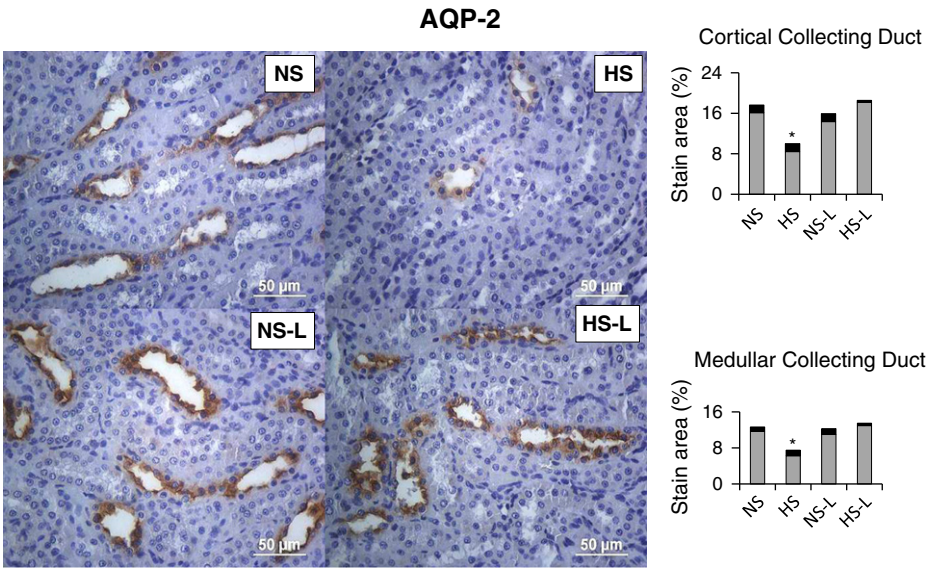


Fig. 3. AQP-2 immunoexpression in renal tissues. Assessment of the immunohistochemical expression of AQP-2 in renal tissue. NS: normosodic diet; HS: high-sodium diet; NS-L: normosodic diet plus losartan; HS-L: high-sodium diet plus losartan. Values are expressed as percentage (%) of the positively stained area ± SEM; n = 5; *p < 0.01 vs. the NS group; †p < 0.01 vs the respective control without losartan. Representative image of positive staining corresponding to AQP-2 expression in the renal medulla (400×).

medulla in the HS group with respect to NS rats. Losartan, which showed no effects in NS rats, prevented the decrease in eNOS staining in the HS-L group, which showed a stain similar to that observed in the NS group.

4. Discussion

The present study constitutes the first report demonstrating that an excess in dietary salt downregulates AQP-1 and AQP-2 levels in proximal tubules and collecting ducts respectively, and that this downregulation is prevented by Ang II-AT1 blockade by losartan. In addition, losartan increased AQP-1 expression in proximal tubules independently of sodium load, promoting the concentration of sodium in the urine. Furthermore, we showed that an excess of salt in the diet also increases renal Ang II expression and develops a profibrogenic response in the kidney, as evidenced by Ang II, TGF-β_1 and α-SMA overexpression in renal tissues. All these alterations were prevented by the administration of the AT1 receptor antagonist, losartan.

Table 2
Renal expression of Ang II, eNOS, TGF-β_1 and α-SMA.

		Ang II (%)	eNOS (%)	TGF-β_1 (%)	α-SMA (no of myofibroblasts/area)
Cortex	NS	16.6 ± 1.8	23.4 ± 2.1	26.9 ± 0.6	4.2 ± 0.8
	HS	44.7 ± 0.8*	11.2 ± 2.2*	48.7 ± 1.1*	33.2 ± 1.3*
	NS-L	20.9 ± 0.9	20.7 ± 3.3	29.1 ± 1.1	16.8 ± 2.4*
	HS-L	17.0 ± 1.1†	28.6 ± 1.5†	24.2 ± 0.8†	18.7 ± 1.9*†
Medulla	NS	15.5 ± 1.6	24.3 ± 1.2	19.3 ± 0.5	10.5 ± 0.9
	HS	35.7 ± 1.1*	10.4 ± 2.3*	36.8 ± 0.8*	127.4 ± 1.9*
	NS-L	13.8 ± 0.8	18.3 ± 3.0	16.5 ± 0.8	22.9 ± 2.7*
	HS-L	17.1 ± 1.1†	24.9 ± 1.3†	17.3 ± 0.7†	19.7 ± 1.4*†

Expression of angiotensin II (Ang II), endothelial nitric oxide synthase (eNOS), transforming growth factor-β_1 (TGF-β_1) and α-smooth muscle actin in the renal cortex and medulla, in the following groups: normosodic (NS), high-sodium (HS), normosodic-losartan (NS-L) and high-sodium-losartan (HS-L). *p < 0.01 vs NS; †p < 0.01 vs respective control without losartan.

In the present study, we observed that Ang II immunoexpression increased in the renal cortex and medulla. This increase could be a result from a greater uptake of circulating Ang II by stimulation of AT1 receptor and/or from de novo generation of intrarenal Ang II, derived from local intrarenal angiotensinogen, produced and secreted by proximal tubular cells [4]. Data in literature are contradictory. Clinical and experimental studies have demonstrated that the excessive salt intake increases oxidative stress, AT1 receptor expression as well as the renal Ang II levels [18–21], whereas AT2 receptors are downregulated [22]. However, Fox et al. reported that, in normal conditions, an increase in salt intake inhibits both, circulating Ang II and the tissue renin–Ang II system [23]. In addition, Lara et al. described that Sprague–Dawley rats subjected to a high salt diet for 2 weeks did not show changes in urinary angiotensinogen excretion although they exhibited enhanced deposition of collagen and increased NADPH oxidase activity in the kidneys [24]. However, it must be pointed out that, the oxidative stress is able to induce conformational molecular changes in the angiotensinogen molecule which facilitates a more rapid generation of

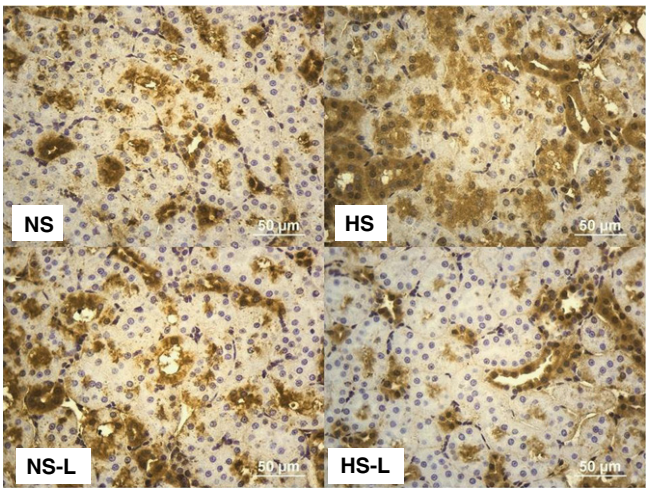


Fig. 4. Ang II immunoexpression. Representative image of positive staining corresponding to Ang II expression (400×). NS: normosodic diet; HS: high-sodium diet; NS-L: normosodic diet plus losartan; HS-L: high-sodium diet plus losartan.

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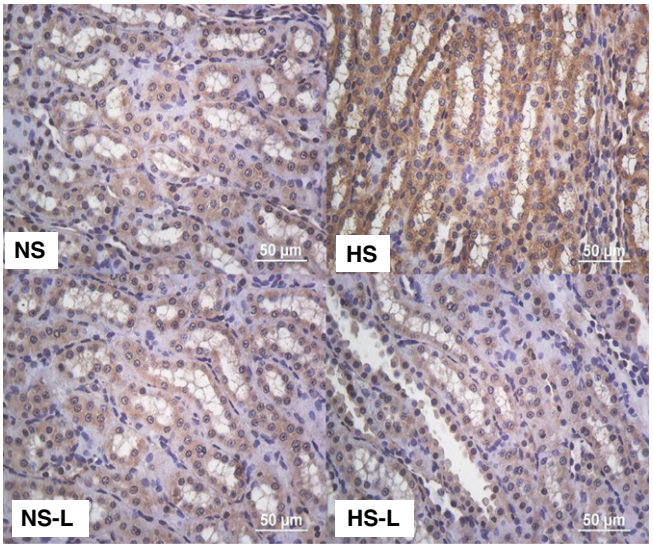


Fig. 5. TGF-β_1 immunoexpression. Representative image of positive staining corresponding to TGF-β_1 expression (400×). NS: normosodic diet; HS: high-sodium diet; NS-L: normosodic diet plus losartan; HS-L: high-sodium diet plus losartan.

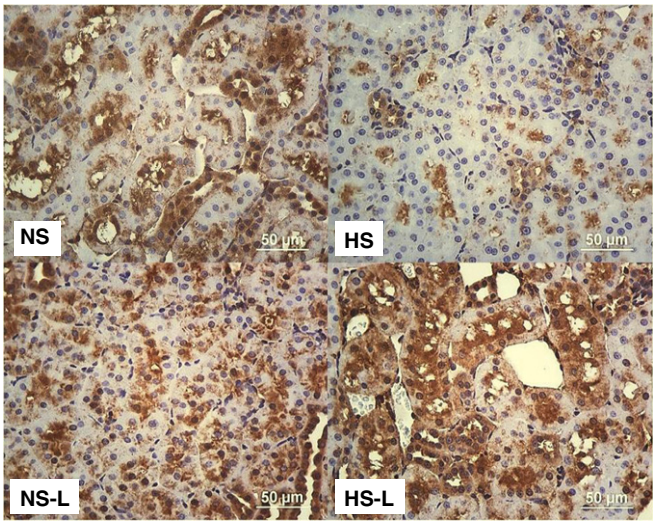


Fig. 7. eNOS immunoexpression. Representative image of positive staining corresponding to eNOS expression (400×). NS: normosodic diet; HS: high-sodium diet; NS-L: normosodic diet plus losartan; HS-L: high-sodium diet plus losartan.

Ang I, when it is exposed to renin [25]. In this order, we have previously demonstrated an increase of NADPH oxidase activity and a profibrogenic response in the kidneys of normal rats fed with a high salt diet, being all these changes prevented by the administration of tempol, a superoxide dismutase mimetic [26].

Since a dietary salt supplement increased blood pressure, we cannot exclude that the lower expression of AQPs found in rats fed with a high-sodium diet, could be a consequence of hypertension. However, Mc Donough et al. reported that acute variations in tensional levels may cause rapid and reversible changes of sodium pump activity, as well as a relocation of the apical sodium transporters [27]. Therefore, we cannot exclude that chronic changes in blood pressure could be implicated in the downregulation of AQP expression in rats fed a high-salt diet.

In order to investigate the effects of renal Ang II, through AT1 receptor stimulation, on AQP regulation in rats fed with a high-salt diet, we analyzed the response caused by losartan. In the HS group, renal Ang II levels were increased and AQP levels were decreased, being these effects reversed by losartan administration. These results could be related to the inhibition of intrarenal rather than circulating Ang II, considering that in our experimental model, circulating Ang II would be decreased by the inhibition of renin secretion by sodium overload. In this order, it has been recently recognized that local renal Ang II is a powerful proinflammatory cytokine and growth factor, implicated in the pathogenesis of progressive renal diseases and the development of tubular–interstitial fibrosis through AT1 receptor binding [28], whereas stimulation of AT2 receptor reduces inflammation and fibrosis in the ischemic kidney through nitric oxide and cGMP production [29]. It is generally admitted as a functional negative crosstalk between AT1 and AT2 receptors in several pathophysiological conditions including hypertension [30]. In addition, AT2 receptor activation may directly antagonize AT1 receptor mediated actions by forming heterodimers with AT1 receptors [31]. Therefore, further studies are needed to elucidate the role of AT1 and AT2 receptors in losartan effects on renal AQP expression.

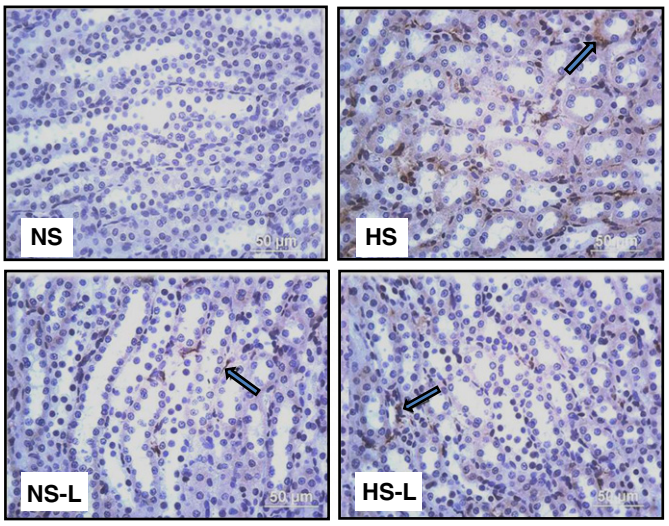


Fig. 6. α-SMA immunoexpression. Representative image of positive staining corresponding to α-SMA expression (400×). NS: normosodic diet; HS: high-sodium diet; NS-L: normosodic diet plus losartan; HS-L: high-sodium diet plus losartan.

In addition, it has been also suggested that the chronic interstitial fibrosis caused by Ang II could be the cause of AQP downregulation and that this fact would be clinically associated with the inability of the kidney to concentrate the urine in different pathological conditions. In this order, Cao et al. [15] have demonstrated that during acute lung injury, Ang II levels are negatively correlated with AQP-1 mRNA in lung tissues. Similarly, Jensen et al. showed that the treatment with the AT1-receptor inhibitor candersartan prevented the decrease in AQP-2 expression in response to the bilateral ureteral obstruction and attenuates the post-obstructive polyuria as well as the renal sodium loss [16]. Moreover, Hasler et al. showed that the activation of NF-kB transcriptional factor by proinflammatory factors such Ang II reduces AQP-2 gene transcription [32]. In the present study, we observed that in the HS group, Ang II and TGF-β_1 immunostaining increased in tubular cells of the renal cortex and medulla, and α-SMA staining increased in the renal interstitium, whereas eNOS was decreased in the renal cortex and medulla. Therefore, since losartan caused a marked decrease of the expression of profibrogenic markers, and prevented changes in eNOS expression, it is possible to suggest that the enhancement of intrarenal Ang II expression in the HS group may be responsible for the downregulation of AQPs in the kidney, as a consequence of a pro-inflammatory or a pro-fibrogenic action. Moreover, the protective action of losartan could not only be due to AT1 blockade, but also to Ang II stimulation on the available AT2 receptors and the response through eNOS–nitric oxide–cGMP signaling. The activation of the AT2 signaling pathway could prevent the proinflammatory response and up-regulate AQPs in renal tissues.

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In addition to preventing AQP-1 downregulation, losartan upregulated AQP-1 expression in the proximal tubule from both the NS-L and HS-L groups, suggesting that this effect was independent of the sodium overload. Further studies are required to elucidate the significance of these observations.

In the present study, a high-salt intake caused a meaningful increase in UV_{Na} and $FE_{Na,}$ without changing the GFR. The simultaneous administration of losartan increased even more UV_{Na} and $FE_{Na,}$ but did not alter the GFR. Conversely, in the control group fed with normosodic diet, losartan caused a decrease in SBP and GFR, but did not modify sodium excretion, thus increasing the FE_{Na}. The control of sodium excretion and blood pressure by Ang II is exerted through multiple intrarenal as well as extrarenal mechanisms. The most important intrarenal effects of Ang II include efferent arteriolar constriction through AT1 receptor stimulation, as well as direct effects on sodium transport. The constrictor effect on efferent arterioles is also important in preventing reductions of GFR in circumstances associated with impaired renal perfusion. Therefore, the fall in blood pressure and the direct effect on efferent arterioles produced by losartan in normal rats could cause reductions in the GFR, as we observed in the present study. Our studies revealed that losartan exerted a natriuretic effect at the tubular level, independent of its hypotensive effect and of GFR changes. Moreover, Ang II overexpression in rats fed with a high-salt diet was accompanied by a marked decrease in eNOS expression in the renal cortex and medulla, being this decrease normalized by losartan treatment. It is well documented that renal AT1 receptors, via activation of renal sodium transporters, produce antinatriuretic effects [27], whereas AT2 receptors activate the nitric oxide/cGMP pathway leading to an increase in urinary sodium excretion [33,34], thus influencing renal Na/fluid homeostasis. Therefore, the natriuretic action of losartan could not only be due to AT1 blockade, but also to Ang II stimulation on the available AT2 receptors. Finally, we observed that losartan increased urinary sodium concentration. In this order, it has been described that AQP-2 expression in the kidney is related to changes in nitric oxide synthase activity [35]. Additionally, it has been shown that the activation of a cGMP-dependent pathway results in AQP-2 insertion to the cell membrane, a fact that can be mediated by different cGMP pathway activators, such as the atrial natriuretic peptide, L-arginine and nitric oxide [36]. Therefore, we can suggest that the decrease of eNOS expression, elicited by increased intrarenal Ang II levels, could be responsible for AQP-2 downregulation in the renal tubules of rats subjected to HS diet, and that losartan administration could exert an antidiuretic effect through Ang II–AT2–nitric oxide–cGMP signaling.

As it is illustrated in Fig. 8, a high salt diet may contribute to increase Ang II expression in renal tubular cells. In addition, intracellular Ang II, via AT1 receptor binding may stimulate cytokine production causing inflammation, fibrosis and downregulating of AQP expression. The AT1 blocker losartan, prevented intrarenal Ang II overexpression and the fibrogenic response, and increased AQP immunoexpression. In contrast, the signaling cascade AT2–eNOS–NO could be suppressed by a high salt diet. These effects were prevented by losartan administration, suggesting that intrarenal Ang II also has a deleterious profibrogenic effect and decreases urinary concentration ability. The fact that losartan also normalized eNOS expression, suggests that its antifibrotic, natriuretic and antidiuretic effects could be partly attributed to unmasked AT2 receptor stimulation by Ang II.

In summary, our findings support the idea that renal Ang II is closely involved in AQP downregulation when an excess of sodium is included in the diet. In addition, AT1 receptor blockade by losartan administration during a dietary salt supplementation could exert beneficial effects, enhancing the ability of the kidney to concentrate the urine.

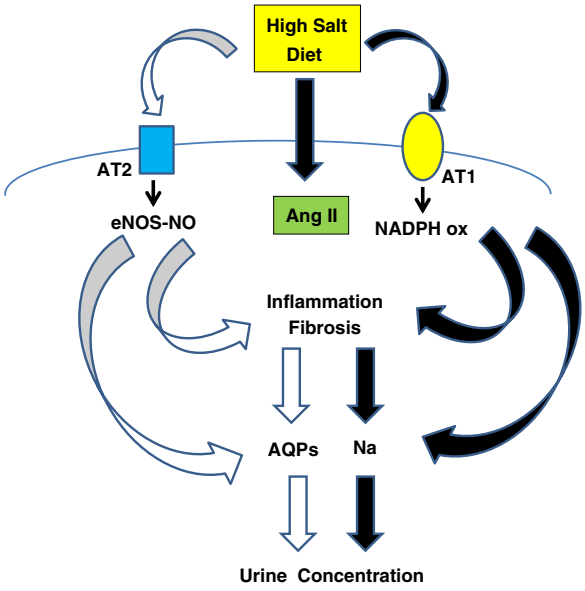


Fig. 8. Schematic figure summarizing our findings. White arrows indicate inhibition; black arrows indicate stimulation. A high salt diet may contribute to increase Ang II expression in renal tubular cells. Intrarenal Ang II, via AT1 receptor binding may stimulate cytokine production causing inflammation and fibrosis, NADPH oxide activation, AQP downregulation and sodium retention, enhancing urine concentration. On the other hand, the signaling cascade AT2–eNOS–NO which normally counterbalances AT1 receptor stimulatory actions could be suppressed by a high salt diet, reinforcing the effects of AT1 stimulation. These effects were prevented by AT1 receptor blockade by losartan administration, suggesting that intrarenal Ang II also has a deleterious profibrogenic effect and decreases urinary concentration ability in the kidney. The fact that losartan also normalized eNOS expression, suggests that its antifibrotic, natriuretic and antidiuretic effects could be partly attributed to unmasked AT2 receptor stimulation by Ang II.

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References

[1] Sabolic I, Valenti G, Verbavatz JM, Van Hoek AN, Verkman AS, Ausiello DA, Brown D. Localization of the CHIP28 water channel in rat kidney. Am J Physiol Cell Physiol 1992;263:C1225–33.
[2] Pallone TL, Edwards A, Ma T, Silldorff EP, Verkman AS. Requirement of aquaporin-1 for NaCl-driven water transport across descending vasa recta. J Clin Invest 2000;105:215–22.
[3] Takata K, Matsuzaki T, Tajika Y, Ablimit A, Hasegawa T. Localization and trafficking of aquaporin 2 in the kidney. Histochem Cell Biol 2008;130(2):197–209.
[4] Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin–angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. Pharmacol Rev 2007;59(3):251–87.
[5] Li XC, Hopfer U, Zhuo JL. AT1 receptor-mediated uptake of angiotensin II and NHE-3 expression in proximal tubule cells through a microtubule-dependent endocytic pathway. Am J Physiol Renal Physiol 2009;297(5):F1342–52.
[6] Hakam AC, Hussain T. Angiotensin II type 2 receptor agonist directly inhibits proximal tubule sodium pump activity in obese but not in lean Zucker rats. Hypertension 2006;47(6):1117–24.
[7] Wintour EM, Earnest L, Alcorn D, Butkus A, Shandley L, Jeyaseelan K. Ovine. AQP1: cDNA cloning, ontogeny, and control of renal gene expression. Pediatr Nephrol 1998;12(7):545–53.
[8] Lee YJ, Song IK, Jang KJ, Nielsen J, Frøkiaer J, Nielsen S, Kwon TH. Increased AQP2 targeting in primary cultured IMCD cells in response to angiotensin II through AT1 receptor. Am J Physiol Renal Physiol 2007;292(1):F340–50.
[9] Wang W, Li C, Summer S, Falk S, Schrier RW. Interaction between vasopressin and angiotensin II in vivo and in vitro: effect on aquaporins and urine concentration. Am J Physiol Renal Physiol 2010;299(3):F577–84.
[10] Bouley R, Palomino Z, Tang SS, Nunes P, Kobori H, Lu HA, Shum WW, Sabolic I, Brown D, Ingelfinger JR, Jung FF. Angiotensin II and hypertonicity modulate proximal tubular aquaporin 1 expression. Am J Physiol Renal Physiol 2009;297(6):F1575–86.
[11] Quan A, Baum M. Regulation of proximal tubule transport by endogenously produced angiotensin II. Nephron 2000;84(2):103–10.
[12] Prieto MC, Navar LG. Collecting duct renin a critical link in angiotensin II: dependent hypertension. In: Frohlich ED, Re R, editors. The local renin–angiotensin aldosterone system. New York: Springer; 2009. p. 133–41.

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[13] Kobori H, Harrison-Bernard LM, Navar LG. Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. Kidney Int 2002;61(2):579–85.
[14] Siragy HM. Angiotensin II compartmentalization within the kidney: effects of salt diet and blood pressure alterations. Curr Opin Nephrol Hypertens 2006;15:50–3.
[15] Cao CS, Yin Q, Huang L, Zhan Z, Yang JB, Xiong HW. Effect of angiotensin II on the expression of aquaporin 1 in lung of rats following acute lung injury. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2010;22(7):426–9.
[16] Jensen AM, Li C, Praetorius HA, Nørregaard R, Frische S, Knepper MA, Nielsen S, Frøkiaer J. Angiotensin II mediates downregulation of aquaporin water channels and key renal sodium transporters in response to urinary tract obstruction. Am J Physiol Renal Physiol 2006;291(5):F1021–32.
[17] Drenjančević-Perić I, Jelaković B, Lombard JH, Kunert MP, Kibel A, Gros M. High-salt diet and hypertension: focus on the renin–angiotensin system. Kidney Blood Press Res 2011;34:1–11.
[18] Chandramohan G, Bai Y, Norris K, Rodriguez-Iturbe B, Vaziri ND. Effects of dietary salt on intrarenal angiotensin system, NAD(P)H oxidase, COX-2, MCP-1 and PAI-1 expressions and NF-kappaB activity in salt-sensitive and -resistant rat kidneys. Am J Nephrol 2008;28(1):158–67.
[19] Williams DE, Prieto MC, Mullins JJ, Navar LG, Mitchell KD. AT1 receptor blockade prevents the increase in blood pressure and the augmentation of intrarenal ANG II levels in hypertensive Cyp1a1-Ren2 transgenic rats fed with a high-salt diet. Am J Med Sci 2010;339(4):356–61.
[20] Fan YY, Baba R, Nagai Y, Miyatake A, Hosomi N, Kimura S, Sun GP, Kohno M, Fujita M, Abe Y, Nishiyama A. Augmentation of intrarenal angiotensin II levels in uni-nephrectomized aldosterone/salt-treated hypertensive rats; renoprotective effects of an ultrahigh dose of olmesartan. Hypertens Res 2006;29(3):169–78.
[21] Susic D, Frohlich ED. Salt consumption and cardiovascular, renal, and hypertensive diseases: clinical and mechanistic aspects. Curr Opin Lipidol 2012;23(1):11–6.
[22] Gonzalez M, Lobos L, Castillo F, Galleguillos L, Lopez NC, Michea L. High-salt diet inhibits expression of angiotensin type 2 receptor in resistance arteries. Hypertension 2005;45(5):853–9.
[23] Fox J, Guan S, Hymel AA, Navar LG. Dietary Na and ACE inhibition effects on renal tissue angiotensin I and II and ACE activity in rats. Am J Physiol 1992;262(5 Pt 2): F902–9.
[24] Lara LS, McCormack M, Semprum-Prieto LC, Shenouda S, Majid DS, Kobori H, Navar LG, Prieto MC. AT1 receptor-mediated augmentation of angiotensinogen, oxidative stress, and inflammation in ANG II-salt hypertension. Am J Physiol Renal Physiol 2012;302(1):F85–94.
[25] Zhou A, Carrell RW, Murphy MP, Wei Z, Yan Y, Stanley PL, Stein PE, Broughton Pipkin F, Read RJ. A redox switch in angiotensinogen modulates angiotensin release. Nature Nov 4 2010;468(7320):108–11.
[26] Rosón MI, Cao G, Della Penna S, Gorzalczany S, Pandolfo M, Cerrudo C, Fernández BE, Toblli JE. High sodium diet promotes a profibrogenic reaction in normal rat kidneys. Effects of Tempol administration. J Nephrol 2011;24(1):119–27.
[27] Mc Donough AA. Mechanisms of proximal tubule sodium transport regulation that link extracellular fluid volume and blood pressure. Am J Physiol Regul Integr Comp Physiol 2010;298:R851–86.
[28] Navar LG, Prieto MC, Satou R, Kobori H. Intrarenal angiotensin II and its contribution to the genesis of chronic hypertension. Curr Opin Pharmacol 2011;11(2): 180–6.
[29] Matavelli LC, Huang J, Siragy HM. Angiotensin AT_2 receptor stimulation inhibits early renal inflammation in renovascular hypertension. Hypertension 2011;57(2):308–13.
[30] Mogi M, Iwai M, Horiuchi M. New insights into the regulation of angiotensin receptors. Curr Opin Nephrol Hypertens 2009;18(2):138–43 [Review].
[31] AbdAlla S, Lother H, Abdel-tawab AM, Quitterer U. The angiotensin II AT2 receptor is an AT1 receptor antagonist. J Biol Chem 2001;276(43):39721–6 [26].
[32] Hasler U, Leroy V, Martin PY, Féraille E. Aquaporin-2 abundance in the renal collecting duct: new insights from cultured cell models. Am J Physiol Renal Physiol 2009;297(1): F10–8.
[33] Sabuhi R, Asghar M, Hussain T. Inhibition of NAD(P)H oxidase potentiates AT2 receptor agonist-induced natriuresis in Sprague–Dawley rats. Am J Physiol Renal Physiol 2010;299(4):F815–20.
[34] Hakam AC, Hussain T. Renal angiotensin II type 2 receptors are upregulated and mediate the candesartan-induced natriuresis/diuresis in obese Zucker rats. Hypertension 2005;45:270–5.
[35] Boone M, Kortenoeven M, Robben JH, Deen PM. Effect of the cGMP pathway on AQP2 expression and translocation: potential implications for nephrogenic diabetes insipidus. Nephrol Dial Transplant 2010;25(1):48–54.
[36] Bouley R, Breton S, Sun T, McLaughlin M, Nsumu NN, Lin HY, Ausiello DA, Brown D. Nitric oxide and atrial natriuretic factor stimulate cGMP-dependent membrane insertion of aquaporin 2 in renal epithelial cells. J Clin Invest 2000;106(9): 1115–26.