Activation of the Renin-Angiotensin System by a Low Salt Diet Does Not Augment Intratubular Angiotensinogen and Angiotensin II in Rats

Weijian Shao, Dale M. Seth, Minolfa C. Prieto, Hiroyuki Kobori and L. Gabriel Navar

Department of Physiology, Hypertension and Renal Center of Excellence,
Tulane University School of Medicine, New Orleans, LA

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Low Salt Doesn’t Augment Intratubular AGT and Ang II in Rats

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Corresponding Author:

Weijian Shao
Department of Physiology, SL39
Tulane University Health Sciences Center
1430 Tulane Avenue
New Orleans, LA 70112
Phone: 504-988-2610
Fax: 504-988-2675
Email: wshao@tulane.edu

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Abstract

In angiotensin II (Ang II) infusion hypertension, there is an augmentation of intratubular angiotensinogen (AGT) and Ang II leading to increased urinary AGT and Ang II excretion rates associated with tissue injury. However, the changes in urinary AGT and Ang II excretion rates and markers of renal injury during physiologically induced stimulation of the renin-angiotensin system (RAS) by a low salt diet remain unclear. Male Sprague-Dawley rats received low salt diet (0.03% NaCl, n = 6) and normal salt diet (0.3% NaCl, n = 6) for 13 days. Low salt diet rats had markedly higher plasma renin activity and plasma Ang II levels. Kidney cortex renin mRNA, kidney AGT mRNA and AGT immunoreactivity were not different; however, medullary renin mRNA, kidney renin content and kidney Ang II levels were significantly elevated by the low salt diet. Kidney renin immunoreactivity was also markedly increased in juxtaglomerular apparatus (JGA) and in cortical and medullary collecting ducts. Urinary AGT excretion rates and urinary Ang II excretion rates were not augmented by the low salt diet. The low salt diet caused mild renal fibrosis in glomeruli and the tubulointerstitium, but no other signs of kidney injury were evident. These results indicate that, in contrast to the response in Ang II infusion hypertension, the elevated plasma and intrarenal Ang II levels caused by physiological stimulation of RAS are not reflected by increased urinary AGT or Ang II excretion rates or the development of renal injury.

Key Words: Urinary Angiotensinogen, Urinary Angiotensin II, Kidney Injury Markers, Renin Expression, Collecting Duct Renin
**Introduction**

Previous studies have demonstrated that chronic Ang II infusions to normal rats significantly increase urinary excretion of endogenous Ang II in a time-dependent manner which is mediated by Ang II type 1 (AT$_1$) receptors [48]. While juxtaglomerular cell and plasma renin activity (PRA) are markedly reduced by Ang II infusions [44; 48], the renin mRNA and protein in principal cells of collecting ducts are increased [10; 44]. Chronic Ang II infusion also results in significant increases in renal expression of AGT mRNA and protein [26; 27]. Furthermore, urinary excretion of AGT is significantly increased in a dose-and time-dependent manner in association with enhancement of intrarenal AGT and Ang II levels [28].

The augmentation of the intrarenal RAS during Ang II infusion hypertension leads to the progressive development of renal injury as evidenced by increased oxidative stress, glomerular and interstitial fibrosis, mesangial expansion, cellular proliferation and macrophage accumulation [33]. These results support the hypothesis that urinary excretion of AGT and/or Ang II serves as an index of intrarenal Ang II production in Ang II infusion hypertension [30; 48], and contribute to the development of renal injury. However, the changes in urinary AGT and Ang II excretion rates during physiologically induced stimulation of the RAS in normal rats remain unclear. In Ang II infused rats, there is significant kidney mesangial expansion, collagen deposition and macrophage infiltration and cell proliferation [33], which may contribute to renal injury. Because these changes are prevented by blockade of the RAS, they have been attributed to being mediated solely by AT$_1$ receptor activation. If this was true, the effects of RAS stimulation by low salt diet on renal histology in normotensive rats should be similar. Low salt diet activates the RAS, increases oxidative stress via NADPH oxidase, attenuates nitric oxide
bioavailability in the heart [51], and increases prorenin receptor (PRR) expression in kidney tissues [20]. However, the overall responses of the intratubular RAS and other markers of renal inflammation and injury have not been assessed.

Low salt diet produces a pronounced stimulation of both circulating and renal RAS, which induces an elevation in PRA associated with increases in Ang I and Ang II levels in plasma, kidneys and other organs throughout the body [6; 22; 23]. Although salt restriction enhances AGT mRNA expression in the kidney [21; 24; 49], there is a decrease in plasma AGT levels which could be due to a higher consumption of AGT by the elevated plasma renin [6; 22; 23]. Because there is little information on the intratubular RAS and development of renal injury, the objective of this study was to evaluate in a comprehensive manner the effects of physiological activation of the RAS by a low salt diet on the intratubular RAS, in particular, as reflected by the urinary AGT and Ang II excretion rates and markers of renal injury in normal rats.

**Materials and Methods**

**Animal Preparation**

The experimental protocol was approved by the Animal Care and Use Committee at Tulane University. Male Sprague-Dawley rats weighing 350 to 380 g (Charles River Laboratories) were housed in metabolic cages and maintained in a temperature-controlled room regulated on a 12-hour light/dark cycle with free access to water. Rats were divided at random into two groups and placed on a normal salt diet (NS group, 0.3% NaCl, Ralston Purina; n = 6) or a low salt diet (LS group, 0.03% NaCl, Dyets; n = 6). Systolic blood pressure (SBP) was measured every three days in conscious rats by tail-cuff plethysmography (IITC Life Science). The rats were
euthanized by conscious decapitation on day 13, and trunk blood was collected and the kidneys were immediately removed, quickly weighed, and homogenized in methanol. The time delay between decapitation and homogenization of the kidneys did not exceed 60 seconds.

Collection and Extraction of Blood, Kidney, and Urine Samples

Trunk blood was collected in chilled tubes containing a mixed inhibitor solution (final concentration: 5 mmol/L of EDTA, 20 µmol/L of pepstatin A, 10 µmol/L of PMSF, 20 µmol/L of enalaprilat, and 1.25 mmol/L of 1,10-phenanthroline). After centrifugation at 4°C for 10 minutes at 1000g, plasma was separated and applied to phenyl-bonded solid-phase extraction columns (Bond-Elut, Varian) that had been prewashed with 90% methanol followed by water. After sample application, each solid-phase extraction column was washed sequentially with water, hexane, and chloroform. Angiotensin peptides were eluted from the solid-phase extraction column with 90% methanol [48]. The eluates were collected, evaporated to dryness under vacuum, and stored at -20°C until radioimmunoassay was performed.

Left kidneys were sectioned for measuring the expression of renin and AGT mRNA levels and immunohistochemistry studies. Renal cortices were dissected from inner medullae under stereomicroscopy and RNase-free conditions. For tissue Ang II measurements, half of the right kidneys were immersed in cold methanol (100%), minced, and homogenized with a polytron tissue tearor immediately after harvesting and weighing. The homogenates were centrifuged, and the supernatants from the kidney homogenates were dried overnight in a vacuum centrifuge. The dried residue was reconstituted in 1 mL of radioimmunoassay buffer (19 mmol/L of monobasic sodium phosphate, 81 mmol/L of dibasic sodium phosphate, 0.05 mol/L of sodium chloride,
0.1% BSA, 0.1% Triton X-100, and 0.01% sodium azide [pH 7.4]). These samples were extracted and evaporated as described above for plasma.

Urine was collected into tubes containing the same mixed inhibitor solution as for the blood. Collection was carried out for 24 hours on the day before low salt diet was started and on days 3, 6, 9, and 12. Urine was centrifuged, and representative 1.0 mL aliquots of the supernatants were extracted and evaporated as described above for plasma.

**Plasma Renin Activity (PRA) and Kidney Renin Content (KRC)**

For PRA determinations, trunk blood was collected in chilled tubes containing EDTA (5 mmol/L). Plasma was separated and stored at -20°C until assayed with a commercially available GammaCoat Plasma Renin Activity 125I RIA kit (DiaSorin) [53]. For KRC assessment, half of right kidneys were immersed in cold KRC homogenization buffer (2.6 mM EDTA, 3.4 mM hydroxyquinoline, 5 mM ammonium acetate, 200 µM PMSF, 0.256 µM dimercaprol), minced, and homogenized. The homogenates were centrifuged and the supernatants were used to generate 1:1,000 dilutions that were spiked with 1 µM synthetic renin tetradecapeptide substrate and the generated Ang I was assayed with the Diasorin PRA RIA kit.

**Plasma and Kidney Ang II**

The reconstituted samples were incubated with a rabbit anti–Ang II serum (Peninsula Laboratories Inc) and 125I-radiolabeled Ang II (Perkin Elmer Life and Analytic Sciences) for 48 hours at 4°C. Bound and free Ang peptides were separated by dextran-coated charcoal, and the supernatants were counted on a computer-linked gamma counter for 3 minutes. The sensitivity of
the Ang II assay was 0.79 fmol. For the Ang II assays, the specific binding was 78.5%, and nonspecific binding was 0.5%.

Urinary Angiotensinogen (uAGT)

Urinary concentrations of AGT were measured using an ELISA kit (Immuno-Biological Laboratories, Minneapolis, MS) as previously described [25; 29] and urinary excretion rates were calculated from the 24 hour volumes collected.

Renal Expression of AGT mRNA

Total RNA was extracted from part of the kidney using an RNeasy Midi Kit (Qiagen, Chatsworth, California, USA). For determination of AGT mRNA, real-time quantitative RT-PCR was then performed as described previously [27; 31].

mRNA Levels of Renin

For reverse transcriptase–polymerase chain reaction (RT-PCR) purposes, first strand cDNA synthesis was performed using 5 μg of total RNA and SuperScript II RNase H-reverse transcriptase system (Invitrogen Life Technologies Co, Carlsbad, Calif). Synthetic specific primers located in exons 1 and 5 of renin 1c gene (sense 5'-ATGCCTCTCTGGGCACTCTT-3' and antisense 5'- GTCAAACTTGCCAGCATGA-3') were used with standard experimental conditions as previously described [43].

Histology and Immunohistochemistry of Kidney Injury Markers
For the renin immunohistochemistry, formalin fixed kidneys were dehydrated in graded concentrations of alcohol, embedded in paraffin blocks, sectioned and mounted onto slides with Vectabond (Vector Laboratories, Inc., Burlingame, CA). Serial kidney sections were immunostained by the immunoperoxidase technique with modifications as described previously [42]. Renin immunolocalization was assessed using a polyclonal anti-rat specific renin antibody raised in rabbit (generously provided by Dr. Tadashi Inagami, Vanderbilt University) and an automatic robot system (DAKO Corp., Carpinteria, CA) which allowed us to apply identical conditions to all slides. The results are expressed in arbitrary units of the relative intensity normalized to the renin immunostaining average of the normal salt group. Immunohistochemical analysis of AGT was performed with a rabbit polyclonal anti-mouse/rat AGT antibody (Immuno-Biological Laboratories Co. Ltd, Takasaki, Japan), using an Autostainer System (DakoCytomation, Glostrup, Denmark). For each rat, the cortical lesions in 20 consecutive microscopic fields were examined, and the intensities of AGT immunoreactivity were calculated and averaged using Image Pro Plus software (Media Cybernetics, Bethesda, MD, USA).

Kidney sections (3 µm), paraffin-embedded, were stained with 1) Masson’s trichrome (Mass Histology, Worcester, MA) for assessment of glomerular and interstitial fibrosis and 2) periodic acid-Schiff (PAS) for the determination of glomerular lesions. Morphometry of glomeruli and the extent of the interstitial fibrotic area were evaluated quantitatively using computerized image analysis, as described [12; 35]. For the immunoexpression of proliferating cell nuclear antigen (PCNA) and CD68, markers of cell proliferation and macrophage infiltration, respectively, monoclonal antibodies for PCNA and CD68 were used at 1:1,000 dilutions [12; 35], respectively. All data were expressed as fold-changes. All of the staining was performed
by a robotic system (Autostainer; Dako, Carpinteria, CA) and analyzed using photomicrographs at 200× or 400× magnification from 20 different microscopic fields per tissue section per animal, and digital images were captured with a DS-U2/L2 USB camera attached to a Nikon Eclipse 50i microscope and an NIS Elements AR version 3.0 software image analyzer in a blinded manner to avoid bias.

Statistical Analysis

Results are expressed as means ± SEM. Data were analyzed by repeated-measures ANOVA with post hoc Newman-Keuls multiple comparison test within each group, and by 1-way ANOVA with post hoc Newman-Keuls multiple comparison test between two groups. We used unpaired t test to analyze two group data. A value of $P<0.05$ was considered statistically significant.

Results

Blood Pressure and Metabolic Parameters

Systolic blood pressures were similar at the onset of the study but were increased slightly in low salt diet rats at day 9 (129 ± 3 vs. 115 ± 3 mmHg). However, SBP values were not statistically different from those in normal salt diet rats by day 12 (118 ± 4 vs. 113 ± 2 mmHg; Figure 1A). Low salt diet induced significant decreases in water intake and urine volume compared with normal salt diet rats (Table 1). Low salt diet decreased urinary sodium excretion rates sharply by day 3 (20 ± 6 vs. 89 ± 16 mmol/24h; $p<0.05$) and trended even lower by day 12 (15 ± 3 vs. 85 ± 18 mmol/24h; $p<0.05$; Figure 1B); however, serum sodium concentrations were not significantly different compared with normal salt diet rats (Table 1).
Plasma Renin Activity and Ang II levels

Plasma renin activity was markedly elevated in low salt diet rats (13.9 ± 2.7 vs. 4.3 ± 0.7 Ang I ng/ml/hr; p< 0.05; Figure 2A). Likewise compared to normal salt diet rats, low salt diet rats also had significantly higher plasma Ang II levels (154 ± 49 vs. 33 ± 10 fmol/ml; p< 0.05; Figure 2B).

Kidney Renin Content, Renin mRNA, AGT mRNA and Ang II levels

The kidney renin content in low salt diet was augmented (83 ± 12 vs. 56 ± 8 Ang I generation ng/hr/mg; p< 0.05; Figure 3A) and, as previously shown [6; 23], the kidney Ang II levels were significantly elevated in the low salt diet rats compared with the normal salt diet rats (435 ± 57 vs. 265 ± 12 fmol/g; p< 0.05; Figure 3B). Low salt diet induced significantly greater expression of kidney medullary renin mRNA (287 ± 71 vs. 101 ± 17 renin/β-actin ratio (% control); p<0.05), but kidney cortex renin mRNA was not significantly higher compared with normal salt diet rats (174 ± 42 vs. 100 ± 21 renin/β-actin ratio (% control); p>0.05; Figure 4A). In addition, the renal AGT mRNA values were not statistically different in low salt and normal salt diet rats (densitometric value/GAPDH mRNA value ratios of 0.6 ± 0.2 vs. 1.0 ± 0.2).

Kidney Renin and AGT Immunoreactivity

Kidney renin immunoreactivity in JGA (17 ± 3 vs. 1.0 ± 0.3 DU; p < 0.01), cortical (4.1 ± 0.9 vs. 1.0 ± 0.3 DU; p < 0.05) and medullary (2.3 ± 0.3 vs. 1.0 ± 0.2 DU; p < 0.01) collecting duct cells in low salt diet were markedly increased compared with normal salt diet rats (Figure 4B). Kidney AGT immunoreactivity in low salt diet was not different from that in normal salt diet rats (9.4 ± 1.5 vs. 10.1 ± 2.2 DU).
Urinary Excretion Rates of AGT and Ang II

Urinary AGT excretion rates (28 ± 3 vs. 55 ± 7 ng/24h; Figure 5A) and urinary Ang II excretion rates (1196 ± 152 vs. 3181 ± 349 fmol/24h; p< 0.05; Figure 5B) in low salt diet rats were not augmented compared with normal salt diet rats. Urinary AGT concentration in low salt diet was not different from that in normal salt diet (2.6 ± 0.1 vs. 3.1 ± 0.7 ng/ml); however, urinary Ang II concentration was significantly lower in low salt diet compared with normal salt diet (94 ± 5 vs. 166 ± 38 fmol/ml; p < 0.05; Table 1).

Kidney Injury Markers

As shown in Figure 6, low salt diet rats did not show any mesangial expansion (0.7 ± 0.03 vs. 1.0 ± 0.02; p < 0.05; Figure 6) nor cell proliferation in either the renal glomeruli (0.9 ± 0.05 vs. 1.0 ± 0.05; Figure 6) or in the tubulointerstitium (0.4 ± 0.03 vs. 1.0 ± 0.04; p < 0.05; Figure 6) compared with normal salt diet rats. Indeed, low salt diet rats had reduced levels of these markers. Low salt diet did not increase macrophage infiltration in cortical glomeruli (1.0 ± 0.05 vs.1.0 ± 0.04), juxtamedullary glomeruli (JM) (0.6 ± 0.05 vs.1.0 ± 0.03; p< 0.05) or the tubulointerstitium (0.5 ± 0.05 vs.1.0 ± 0.08; p < 0.05; Figure 7). However, the low salt diet rats did appear to have a mild renal fibrosis in glomeruli (2.1 ± 0.11 vs. 1.0 ± 0.07; p < 0.05) and the tubulointerstitium (1.4 ± 0.2 vs. 0.9 ± 0.1; p < 0.05; Figure 8).

Discussion

The RAS is involved in the regulation of arterial pressure and electrolyte balance through the coordinated functions of both systemic and tissue systems [15; 37; 39]. One of the most
important physiological functions is to guard against loss of extracellular fluid sodium and volume by responding with a robust increase in renin secretion during low salt intake [6; 23]. Indeed, a few reports have shown that RAS activation by a low salt diet may actually increase blood pressure slightly in normotensive rats without a genetic predisposition for high blood pressure, such as in two-kidney Goldblatt rats [36], uninephrectomized rats [52], and normotensive rats [19]. These studies are consistent with our results that blood pressure in the low salt group was slightly elevated. The elevated blood pressure in low salt diet rats can be reduced by angiotensin-converting enzyme (ACE) inhibitor [16] indicating that the elevation of blood pressure induced by low salt diet is dependant on activation of the RAS. In uninephrectomized rats, denervation of the remaining kidney markedly attenuated the rise in both the blood pressure and PRA produced by the low salt diet, suggesting that the sympathetic nervous system is activated [52]. Thus, the stimulation of renin release and increased sympathetic nervous system activity may both contribute to the slightly elevated blood pressure by low salt diet which collectively may be responsible for the modest fibrosis which was observed in the low salt group.

In addition to increased renin release, the increased Ang II caused by a low salt diet stimulates aldosterone formation and release leading to further stimulation of sodium reabsorption and decreased urinary sodium excretion rates as reflected by our data. Low salt diet also increases the expression of various Na\(^+\) transporters in tubular cells, thus augmenting the overall renal sodium reabsorption capacity [8; 34]. Ingert et al. reported that intact plasma AGT levels were decreased in low salt diet rats due to a higher consumption of AGT by the elevated circulating renin [23]. Our data show that renin mRNA in the renal medulla was significantly elevated in
low salt diet rats, but there was no change in the renal cortex compared with normal salt diet.

Some studies also reported that the expression of renin mRNA is augmented by low salt diet [21; 49]. Overnight sodium restriction led to a marked increase in connecting tubule immunoreactive renin, but there was no significant change in juxtaglomerular apparatus (JGA) renin; however, longer periods of sodium restriction stimulated renin expression in JGA [46]. In the present study, the total kidney renin content was significantly elevated in low salt rats and renin immunoreactivity was also markedly increased in JGA cells and in collecting ducts from cortex and medulla. In contrast, in Ang II infused rats, renin immunoreactivity is suppressed in JGA cells, but the cortical and medullary collecting duct cells have increased renin immunoreactivity [43]. The increased renal Ang II stimulates renin expression in collecting ducts [43]. In low salt diet rats, the increased renin activity in JG cells and collecting ducts may act cooperatively to augment the intrarenal Ang II levels.

Several studies report that low salt diet does not influence the expression of hepatic AGT mRNA but the renal AGT expression with low salt had not been reported [21; 41]. It is known that renal AGT is expressed in proximal tubular (PT) cells [21; 45] and is secreted into luminal fluid [46]. The action of filtered renin of systemic origin or of distal nephron renin secretion on AGT secreted locally may produce a high luminal concentration of intrarenal Ang II [38; 43], which further augments sodium reabsorption. Although Ang II levels in the kidney were increased in the low salt diet rats, the increases are less when compared to the increases in Ang II levels in Ang II infused rats [48]. The increases of Ang II levels in low salt diet rats in our study are slightly lower in the plasma and in the kidney when compared with previous data in Ang II infused rats [37; 48; 55]. Some studies have shown that the expression of AGT mRNA is
augmented by low salt diet [21; 49], but others have reported no changes in AGT mRNA in low
salt diet [32; 41]. Our data indicate that the expression of AGT mRNA and AGT
immunoreactivity in low salt diet rats were not different from that in rats fed a normal salt diet.
Ingert et al. [22; 23] reported that low salt diet significantly decreased the AGT levels in the
kidney which was also regulated by AT1 blocker, but Lantelme et al. showed no difference in
AGT protein expression [32]. Although low salt diet and Ang II infusion both can activate the
intrarenal RAS, only Ang II infusion results in increases in renal expression of AGT mRNA [27]
and protein [26]. Thus, urinary excretion of AGT and Ang II in Ang II infused rats is
significantly increased in association with enhancement of intrarenal AGT and Ang II levels [28; 48],
while the moderately elevated intrarenal Ang II levels caused by low salt diet do not stimulate urinary excretion of AGT and Ang II. Lantelme et al. [32] also reported that low salt
diet does not increase urinary excretion rates of AGT in CD and C57BL/6 mice. Because a low
salt diet also increases renal tubular expression of the prorenin receptors [20], the elevated
prorenin receptors may increase binding of prorenin and renin which induces an increase in the
catalytic efficiency of AGT cleavage, therefore facilitating Ang II generation [20; 40]. Other
reports indicate that urinary excretion of AGT reflects net tubular flow, rather than systemic
RAS activity or glomerular filtration rate (GFR) during sodium restriction [9; 32]. Although
urinary excretion rates of AGT and Ang II reflect intrarenal production of AGT and Ang II in
Ang II-infused hypertension [28; 48], under physiological conditions, this relationship may be
obscured by the extent of tubular reabsorption of AGT and Ang II [32; 55]. Also, low tubular
flow during salt restriction may increase the duration of exposure to degradative sites thus
reducing uAGT and Ang II. The proximal tubular reabsorptive function and metabolism of these
peptides may play a more important role than does the peptide production rate of the proximal
tubular cells. In low salt diet rats, renal expression of AT$_{1A}$ receptor mRNA was significantly increased [5; 24]. The upregulated AT$_1$ receptors may cause more Ang II uptake from the luminal fluid into the protected compartment endosomes that then prevent degradation and maintain a higher level of intrarenal Ang II [55], which may also contribute to the reduced urinary Ang II excretion rate and urinary Ang II concentrations. Thus, the lower urinary levels could have been due to greater degradation and/or uptake of the peptide as a consequence of the slower transit time along the nephrons.

Although slight renal fibrosis was observed in low salt diet rats, it represents only mild renal injury. The extent of renal fibrosis in the glomeruli is 4 fold higher in rats fed a high salt diet and 5 fold higher in Ang II infused rats when compared to our low salt data [33]. Low salt diet activates RAS, stimulates renin release and upregulates renal expression of the PRR via cGMP-PKG signaling pathways [20]. In addition to the direct effects of increased AT$_1$ receptor activation, the elevated renin and prorenin may directly promote fibrosis through stimulation of PRR via a Nox4-dependent mechanism [3]. These studies indicate that singular increases in RAS intrarenal activity is not sufficient to cause marked renal injury as observed with chronic Ang II infusions. It appears that additional co-existent factors associated with the increased arterial pressure such as increased oxidative stress, cytokines or immunologic and inflammatory factors may be needed to cause more overt renal injury [1; 7; 11; 13; 17; 47; 54]. Low salt diet induces the activation of RAS and the elevated Ang II through its interaction with AT$_1$ receptor activates NADPH oxidase to increase superoxide production and reduce nitric oxide bioactivity [14; 51], which increases oxidative stress [51]. Thus, both sodium restriction by activation of RAS and high salt intake may increase reactive oxygen species (ROS) production that is
considered an important mechanism of organ injury [33]. Accordingly inappropriate stimulation of the intrarenal RAS may be an important contributor to the development of renal injury.

In conclusion, the physiological stimulation of the intrarenal RAS by a low salt diet does not lead to augmentation of the tubular AGT or Ang II levels as occurs in Ang II-infusion hypertension. Thus, the increased urinary AGT and Ang II excretion rates that occur in the hypertensive models appear to be primarily a biomarker of pathological stimulation of the intrarenal RAS and may act synergistically with associated oxidative and inflammatory co-factors to cause hypertension and progressive development of renal injury.

**Perspective**

Population studies have shown that a moderate reduction in salt intake can decrease blood pressure in both hypertensive and non-hypertensive subjects [2]. However, extreme sodium restriction activates the sympathetic system and markedly stimulates the RAS. Thus, it is important that moderate reductions in salt intake should be considered optimal to sustain the reduced blood pressure and prevent organ injury. The long-term moderate reduction of salt intake in the general population leads to significant reduction in cardiovascular events by 25% [4] and potentiates the protective effects of ACE inhibitors and AT1 receptor blockers [18; 50].

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Disclosures

None.


18. Heerspink HJ, Holtkamp FA, Parving HH, Navis GJ, Lewis JB, Ritz E, de Graeff PA and de ZD. Moderation of dietary sodium potentiates the renal and


Legends

Figure 1. 1A, Comparison of systolic blood pressures (SBPs) between low salt diet (n=6) and normal salt diet rats (n=6). 1B, Comparison of urinary sodium excretion rates in low salt diet (n=6) and normal salt diet rats (n=6). Values are means ± SEM. *p< 0.05 vs. normal salt diet rats.

Figure 2. 2A, Comparison of plasma renin activity in low salt diet (n=6) and normal salt diet rats (n=6). 2B, Comparison of plasma Ang II in low salt diet (n=6) and normal salt diet rats (n=6). Values are means ± SEM. *p< 0.05 vs. normal salt diet rats.

Figure 3. 3A, Comparison of kidney renin content in low salt diet (n=6) and normal salt diet rats (n=6). 3B, Comparison of kidney Ang II in low salt diet (n=6) and normal salt diet rats (n=6). Values are means ± SEM. *p< 0.05 vs. normal salt diet rats.

Figure 4. 4A, Comparison of kidney cortex and medulla renin / β actin mRNA (% control) in low salt diet (n=6) and normal salt diet rats (n=6). 4B, Quantification of the intensity of kidney renin immunoreactivity in normal salt diet (A and C, n=6) and low salt diet (B and D, n=6) rats. B shows increased renin immunoreactivity in JG renin and cortical collecting duct cells in low salt diet in comparison to normal salt diet rats (A). D shows increased renin immunoreactivity in medullary collecting duct cells in low salt diet in comparison to normal salt diet rats (C). E, F and G are densitometric analyses of the renin intensity immunoreactivity in JG renin, cortical collecting duct renin and medullary collecting duct renin. Values are means ± SEM. *p< 0.05 vs. normal salt diet rats.
Figure 5. 5A, Comparison of urinary AGT excretion rates in low salt diet (n=6) and normal salt diet rats (n=6). 5B, Comparison of urinary Ang II excretion rates in low salt diet (n=6) and normal salt diet rats (n=6). Values are means ± SEM. *p< 0.05 vs. normal salt diet rats.

Figure 6. Representative photomicrographs of glomerular expansion in normal salt diet (A) and low salt diet (B) rats and image analysis (C). Proliferative cell nuclei antigen (PCNA) was photomicrographed in glomeruli (D – NS, E – LS, F – Image analysis) and also in the tubulointerstitium (G – NS, H – LS, I – Image analysis). Values are means ± SEM. *p< 0.05 vs. normal salt diet rats.

Figure 7. Kidney immunoexpression of CD-68 as a marker of macrophages in cortical glomeruli ( A - NS, B – LS, C – Image analysis), juxtamedullary glomeruli (D - NS, E – LS, F – Image analysis) and the tubulointerstitium (G - NS, H – LS, I – Image analysis). Values are means ± SEM. *p< 0.05 vs. normal salt diet rats.

Figure 8. Representative photomicrographs of collagen deposition in glomeruli (A – NS, B – LS, C – Image analysis) and the tubulointerstitium (D – NS, E – LS, F – Image analysis). Values are means ± SEM. *p< 0.05 vs. normal salt diet rats.
Table 1. Results of metabolic parameters on low and normal salt diet for 13 days

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<th>Parameters</th>
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<th>Low salt diet</th>
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<tr>
<td>Body weight (g)</td>
<td>496.7 ± 19.2</td>
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<td>Water intake (ml/day)</td>
<td>41.3 ± 3.1</td>
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<td>Hematocrit (%)</td>
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<td>Serum sodium (mmol/L)</td>
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<td>Urinary AGT concentration (ng/ml)</td>
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<td>Urinary Ang II concentration (fmol/ml)</td>
<td>165.6 ± 37.8</td>
<td>93.8 ± 5.2 *</td>
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Table 1. Values are means± SEM. *p < 0.05 vs. normal salt diet.
Figure 1

1A

[Graph showing systolic blood pressure (mmHg) over days for normal salt diet and low salt diet.]

1B

[Graph showing Na⁺ excretion rates (mmol/24hr) over days for normal salt diet and low salt diet.]
Figure 2

2A

![Bar chart showing Plasma Renin Activity: Normal salt diet vs Low salt diet.](image)

2B

![Bar chart showing Plasma Ang II: Normal salt diet vs Low salt diet.](image)
Figure 3

3A

![Bar chart showing kidney Renin content in Normal Salt and Low salt conditions.](chart1.png)

3B

![Bar chart showing kidney Ang II levels in Normal salt diet and Low salt diet conditions.](chart2.png)
Figure 4

4A

Kidney Renin/β-actin mRNA

% Control

Cortex | Medulla

| NS | LS | NS | LS |

0 | 100 | 200 | 300 |

4B

Renin

Normal salt diet | Low salt diet

| 0 | 5 | 10 | 15 | 20 |

Relative Densitometry (arbitrary units)

Cortex Medulla
Figure 5

5A

[Graph showing Urinary AGT Excretion Rates with days on the x-axis and ng/24hr on the y-axis. The graph compares Normal salt diet and Low salt diet conditions.

5B

[Graph showing Urinary Ang II Excretion Rates with days on the x-axis and fmol/24hr on the y-axis. The graph compares Normal salt diet and Low salt diet conditions. The graph includes asterisks (*) indicating significance levels.]
Figure 6

Normal Salt  Low Salt

A  B

D  E

G  H

Glomerular Expansion

Fold Changes

0.0  0.4  0.8  1.2

Normal salt diet  Low salt diet

*  *

Glomerular PCNA

Fold Changes

0.0  0.4  0.8  1.2

Normal salt diet  Low salt diet

Tubulointerstitium PCNA

Fold Changes

0.0  0.4  0.8  1.2

Normal salt diet  Low salt diet
Normal Salt  Low Salt

Macrophages Infiltration (CD-68)

Cortical Glomeruli

Juxtamedullary Glomeruli

Tubulointerstitium

* Indicates statistical significance.
Figure 8

Glomerular Fibrosis

Tubulointerstitium Fibrosis

Normal Salt

Low Salt

A

B

C

D

E

F

Fold Changes

Normal salt diet

Low salt diet

*