Intrarenal Renin-Angiotensin System Is Upregulated in Experimental Model of Progressive Renal Disease Induced by Chronic Inhibition of Nitric Oxide Synthesis

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Abstract. Locally generated angiotensin II (AngII) may be involved in the pathogenic mechanisms of chronic renal diseases. Renal expression of AngII and other components of the renin-angiotensin system (RAS) were analyzed by immunohistochemistry and Western blot in a model of chronic progressive nephropathy induced by inhibition of nitric oxide synthesis. Renal injury was evaluated by histology and albumin excretion. Systemic RAS status was evaluated through plasma renin activity (PRA) and plasma AngII concentration. In addition, the effects of enalapril, losartan, and mycophenolate mofetil (MMF) on AngII expression in animals with chronic renal disease was also analyzed. Plasma renin activity and plasma AngII were not different between rats with nephropathy and controls (2.08 ± 0.7 versus 2.03 ± 0.5 ng/ml/h and 94.3 ± 18 versus 78.9 ± 16 fmol/ml, respectively). However, rats with chronic progressive nephropathy showed augmented renal content of angiotensinogen protein (13.5 ± 3.5 versus 2.2 ± 0.4 pixels in control rats; P < 0.05), enhanced expression of cathepsin D—a renin-like enzyme—in cortical collecting tubules (103.5 ± 27.0 versus 66.2 ± 3.6 cells/mm² in controls; P < 0.01), and increased expression of AT1 receptor in interstitium (54.7 ± 7.8 versus 1.3 ± 0.4 cells/mm² in controls; P < 0.001). Kidney angiotensin-converting enzyme content did not differ among the groups. Notably, an increased number of interstitial cells expressing AngII was detected in the renal interstitium (9.5 ± 1.6 versus 1.7 ± 0.6 cells/mm² in controls; P < 0.05). Rats treated with Nω-nitro-L-arginine-methyl-ether and losartan presented a decreased local AngII formation, in contrast to its known effect on plasma AngII. Moreover, mycophenolate mofetil lowered interstitial AngII expression, suggesting that inflammatory signaling may be involved in interstitial AngII generation. This study demonstrates the up-regulation of local RAS in the kidney in a model of chronic progressive nephropathy.

Angiotensin II (AngII), the major effector of renin-angiotensin system (RAS), participates in a series of physiologic and pathophysiologic events, particularly in the kidney (1–4). AngII is involved in the progression of chronic renal disease both in clinical and in experimental models, and renoprotection may be conferred by the suppression of RAS activity (5–8). The beneficial effects of RAS blockers may be a consequence of the reduction of intraglomerular capillary pressure (6) and this local production of AngII and other RAS components in renal tissue of rats with chronic progressive nephropathy. This study demonstrates the up-regulation of local RAS in the kidney in a model of chronic progressive nephropathy.
Materials and Methods

Experimental Groups

To evaluate the systemic and the intrarenal RAS, male adult Wistar rats were divided into three major experimental groups and followed for 30 d: the NAME group consisted of rats that received 200 mg/L L-NAME in their drinking water and a high-salt diet (8.4% NaCl) to induce chronic progressive renal disease (n = 22). The control group included rats receiving a normal-salt diet (3.2% NaCl) (n = 10), and the HS group consisted of rats receiving only the high-salt diet (n = 10).

To analyze the regulation of intrarenal AngII generation, additional groups consisted of rats with progressive renal disease induced by L-NAME plus high-salt diet were treated with enalapril, losartan, or MMF and followed for 15 and 30 d. These animals were divided into three groups: the NAME+ENA group (n = 20), including NAME rats treated with enalapril 60 mg/L in drinking water; the NAME+LOS group (n = 10), consisting of NAME rats receiving losartan 200 mg/L in drinking water; and the NAME+MMF group (n = 14), composed of NAME rats receiving MMF 10 mg/kg/d by gavage. All animals received tap water ad libitum. The experimental procedures were conducted in accordance with our institutional guidelines.

Systolic Blood Pressure (SBP) and Urinary Albumin Excretion

SBP was assessed at 15 and 30 d by systolic blood manometry (Harvard apparatus; Eden Bridge). Twenty-four-hour urinary albumin excretion was assessed after 15 and 30 d by radial immunodiffusion.

Evaluation of Systemic RAS: Plasma Renin Activity (PRA) and Plasma AngII Concentration

Systemic RAS activity was evaluated from PRA and plasma AngII concentrations. To perform these measurements, 5 to 10 rats from the control, HS, and NAME groups accompanied for 30 d were used to detect the expression of RAS components. The following primary antibodies were used: polyclonal rabbit anti-renin (from University of Tsukuba, Japan), polyclonal sheep anti-angiotensinogen (from University of Queensland, Australia), monoclonal mouse anti-ACE (anti CD143, clone 9B9; from Chemicon), polyclonal rabbit anti-AT1 receptor (from RDI), polyclonal rabbit anti-AngII (from Peninsula), and polyclonal rabbit anti—cathepsin D (from Dako). Sections were subjected to microwave irradiation in citrate buffer to enhance antigen retrieval and incubated with the primary antibodies overnight at 4°C in a humidified chamber. After rinsing, the slides were incubated either with rat-adsorbed biotinylated anti-mouse IgG (for ACE) (Vector Labs), biotinylated anti-sheep IgG (for angiotensinogen) (Vector Labs), or biotinylated anti-rabbit IgG (for all other polyclonal antibodies) (Vector Labs). After rinsing, the sections were incubated with the streptavidin-biotin-alkaline phosphate complex (Dako). Finally, sections were incubated with a freshly prepared substrate, consisting of naphthol-AS-MX-Phosphate (Sigma Chemical) and fast red dye (Sigma).

For ACE identification, the kidneys obtained from rats killed by decapitation were snap-frozen in liquid nitrogen and stored at −70°C until processing. Five-micron-thick cryostat renal sections were fixed in cold acetone and then submitted to the indirect streptavidin-biotin alkaline phosphatase method described above, without microwave treatment.

Optimal working dilutions of the primary antibodies were previously determined by titration experiments. Negative control experiments for all antigens were performed by omitting the incubation with the primary antibodies. Additional negative control consisting of incubation of sections with nonspecific Ig (Sigma) was performed.

For quantification of AngII, AT1 receptor, and cathepsin D expression in renal cortex, the number of positively stained cells was counted in 25 consecutive microscopic fields, and the results were expressed as the mean number of stained cells per square millimeter. Quantification of renin expression was performed measuring the ratio of the number of arterioles that stained for renin in a whole section to the absolute number of glomeruli in the same section. Angiotensinogen and ACE were only qualitatively assessed by immunohistochemistry. Quantification of these two components was performed by Western blot.
Western Blot

Proteins were extracted from frozen kidneys after homogenization with a protease inhibitor and quantified by the Bradford method. For angiotensinogen detection, 200 μg of kidney protein samples was electrophoretically separated and transferred to a nitrocellulose membrane (Bio-Rad Laboratories). Blots were incubated with a monoclonal mouse anti-angiotensinogen (RDI) and horseradish-peroxidase conjugated secondary antibody (goat anti-mouse; Santa Cruz Biotechnology). Detection was accomplished with enhanced chemiluminescence (ECL system; Amersham Pharmacia Biotech).

For ACE detection, minor modifications were adopted. Kidney protein aliquots of 60 μg were used. The electrophoresis was performed on a 7.5% slab gel in presence of SDS with 60 μl of denatured and reduced protein. Electrophoresis was performed for 28 min at constant voltage (150 V) with a nitrocellulose membrane (Hybond ECL; Amersham Pharmacia). The membrane was incubated in 5% of blockade solution for 6 h before overnight incubation at 4°C with anti-ACE monoclonal antibody. Subsequent steps were performed with the streptavidin/phosphatase alkaline system (Amersham Pharmacia) as recommended by the manufacturer (Bio-Rad). Western blot analysis was performed in animals observed for 30 d.

ACE Activity

Kidney tissues from the three groups obtained at 30 d were homogenized as described previously (25) in 400 mM phosphate buffer, pH 7.2, containing 340 mM sucrose, 900 mM NaCl (1 g tissue : 10 ml buffer) and the inhibitors p-OHMB (0.1 mM/ml homogenate) and PMSF (100 mM/ml homogenate). The homogenates were centrifuged at 3000 rpm for 10 min and the supernatants frozen at -70°C until used for the experiments.

The ACE catalytic activity was determined fluorometrically. Briefly, an aliquot of homogenate was incubated with 200 μl of assay solution with Hippuryl-L-His-Leu (5 mM) or Z-Phe-His-Leu (1 mM) in 100 mM potassium buffer pH 8.3, containing 300 mM NaCl and 0.1 mM ZnSO4 for 10 min at 37°C. The enzymatic reaction was stopped by the addition of 1.5 ml of 0.28 N NaOH and incubated with 100 μl of o-phthaldialdehyde (20 mg/ml) in methanol during 10 min. The fluorescence reaction was stopped by the addition of 200 μl of 3N HCl. The dipeptide liberated His-Leu was measured fluorometrically (360 nm excitation and 500 nm emission) with a Hitachi fluorometer.

Statistical Analyses

Data are presented as mean ± SEM. One-way ANOVA with pairwise comparisons according to the Newman-Keuls formulation was used in this study. Whenever a nonnormal distribution was found the equivalent nonparametric test, Kruskal-Wallis test with Dunn’s posttest was used; P levels of 0.05 or less were considered significant.

Results

Characterization of Chronic Renal Disease

The presence and the severity of renal disease, analyzed by SBP, albumin excretion, and histology, were evaluated in animals followed for 30 d from the NAME, control, and HS groups. Body weight for all groups was identical at the initiation of the study, but animals with chronic nephropathy gained weight at a slower pace (data not shown). As expected, rats in the NAME group developed severe hypertension. SBP was significantly elevated compared with control and HS groups (Table 1). Albumin excretion rate was markedly increased in

<p>| Table 1. Systolic BP, urinary albumin excretion, and histological parametersa |
|--------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>SBP (mmHg)</th>
<th>Ualb V (mg/d)</th>
<th>Glomerulosclerosis Index</th>
<th>Collapsed Glomeruli</th>
<th>Interstitial Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>150 ± 3</td>
<td>2.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>HS (n = 10)</td>
<td>150 ± 3</td>
<td>2.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>NAME (n = 10)</td>
<td>150 ± 3</td>
<td>2.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>NAME + ENA (n = 20)</td>
<td>150 ± 3</td>
<td>2.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>NAME + LOS (n = 10)</td>
<td>150 ± 3</td>
<td>2.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.05</td>
</tr>
<tr>
<td>NAME + MMF (n = 14)</td>
<td>150 ± 3</td>
<td>2.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.05</td>
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Results are expressed as mean ± SEM. SBP, systolic BP; Ualb V, daily urinary albumin excretion rate. Glomerulosclerosis index is presented as % of sclerotic glomeruli. Collapsed glomeruli are represented as the ratio of abnormal glomeruli to total number of glomeruli in one section. Interstitial area is represented as the fraction of cortical area occupied by interstitium. 

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the NAME group (115 ± 18 mg/24h) compared with the control and HS groups.

The glomerulosclerosis index was significantly higher in the NAME group compared with the control and HS groups. Collapsed glomeruli were more frequently observed in the NAME group compared with the control and HS groups. Rats in the NAME group also showed interstitial injury, characterized as interstitial expansion. The fraction of cortical interstitium occupied by interstitial tissue was markedly increased in the NAME group compared with the control and HS groups.

**Systemic RAS**

The results of PRA and AngII concentrations are shown in Figure 1. PRA was not different between the control and NAME groups (2.03 ± 0.5 and 2.08 ± 0.7 ng/ml/h, respectively; NS). As expected, PRA was suppressed in HS group (0.05 ± 0.03 ng/ml/h) compared with the control and NAME groups (P < 0.05 versus control and NAME). The values of plasma AngII concentration among the control, HS, and NAME groups did not differ (78.9 ± 16 fmol/ml in control group and 67.0 ± 19 fmol/ml in HS group, and 94.3 ± 18 fmol/ml in NAME group; NS).

**Characterization of Intrarenal RAS**

The presence of intrarenal RAS components was analyzed by immunohistochemistry and Western blot in animals with experimental chronic renal disease (NAME group) and in the control and HS groups. The results are presented according to the component.

**Angiotensinogen**

Angiotensinogen expression was detected in tubular epithelial cells in all segments of proximal tubules in animals with chronic renal disease (Figure 2A). However, it was not observed in any other kidney structure. Because all proximal tubular cells equally stained for angiotensinogen, it was not possible to quantify its expression by immunohistochemistry. By use of Western blot, two different specific bands, at 52 and 64 kD, were demonstrated (Figure 3A). Rats with chronic progressive nephropathy (NAME group) exhibited an increase in the density of both bands compared with the control and HS groups (at 52 kD: 13.5 ± 3.5 pixels in the NAME group versus 2.2 ± 0.4 and 2.5 ± 0.2 pixels in the control and HS groups, respectively; P < 0.05; at 64 kD: 10.4 ± 1.6 pixels in the NAME group versus 2.6 ± 0.4 and 4.5 ± 0.4 in the control and

![Figure 1. Quantification of the systemic activity of the renin-angiotensin system. Plasma renin activity (PRA) and plasma angiotensin II (AngII) levels in control, high-salt diet (HS), and Nω-nitro-L-arginine-methyl-ester–treated (NAME) groups.](image-url)
HS groups, respectively; \( P < 0.05 \). Quantification of Western blot results is shown in Figure 3B.

**Renin**

Renin was detected exclusively in the glomerular afferent arterioles (Figure 2B). Faint staining of tubules was observed, but it was not a consistent pattern. The results of renin expression are presented as the ratio of the number of afferent arterioles stained for renin to the absolute number of glomeruli in the whole section. Animals receiving high-salt diet, including rats with chronic renal disease (NAME group), and from the HS group had a lower frequency of stained arterioles (22\% ± 3\% and 10\% ± 2\%, respectively) compared with the control group (40\% ± 2\%; \( P < 0.05 \)).

**Cathepsin D**

Cathepsin D, the alternative enzyme to renin, was detected in the distal nephron, particularly in the cortical collecting tubules (Figure 2C). However, only some cells of these tubules stained for cathepsin. The quantification of positively stained cells disclosed that the NAME group had a higher cathepsin D expression (104.7 ± 3.7 cells/mm\(^2\)) compared with the control and HS groups (66.2 ± 3.5 and 51.1 ± 5.5 in control and HS, respectively; \( P < 0.05 \)).

**ACE**

ACE expression was observed in proximal tubules, particularly in the brush border and also in endothelium of large vessels (Figure 2D). Western blot experiments demonstrated two different isoforms, at 69 (N-domain ACE) and 139 kD (somatic ACE) (Figure 4A). However, no difference was observed among the different groups (control, HS, and NAME) for both isoforms. Rats with chronic progressive nephropathy (NAME group) exhibited the same value in the density of both bands compared with groups the control and HS groups (at 139 kD).
kD: 623 ± 39 pixels in the NAME group versus 762 ± 88 and 692 ± 101 pixels in the control and HS groups, respectively; NS; at 69 kD: 712 ± 88 pixels in the NAME group versus 588 ± 104 and 745 ± 28 in the control and HS groups; NS).

The enzymatic activity of tissues analyzed by Hippuryl-L-His-Leu substrate specific for the C-domain portion of ACE showed no difference among the three groups (control, 1.28 ± 0.1; HS, 1.0 ± 0.1; and NAME, 0.9 ± 0.16 mU/μg protein). However, the analysis of ACE activity by Z-Phe-His-Leu (Figure 4B)—that is, a substrate described with better specificity for N-domain portion of ACE—demonstrated a significantly lower activity of ACE in the NAME group compared with the control and HS groups (1.52 ± 0.18 versus 2.49 ± 0.12 and 2.11 ± 0.17 mU/μg protein, respectively; P < 0.01 and P < 0.05). The difference found when Z-Phe-His-Leu was used could be attributed to the decrease of N-domain ACE activity detected when this substrate is used.

**AngII**

In normal kidneys and in kidneys obtained from rats that received high-salt diet, AngII was detected almost exclusively in glomerular arterioles. Interstitial or glomerular cells also expressed AngII, but less consistently. However, in rats with chronic progressive nephropathy, AngII expression was de-
ected in the afferent arterioles and also in mononuclear cells present in renal cortical interstitium, and this expression paralleled the severity of the renal damage. Interstitial AngII+ cells were particularly observed surrounding damaged vessels and glomeruli (Figure 2E). The number of positively stained cells for AngII in cortical interstitium was 1.7 ± 0.6 cell/mm² in the control group and 1.0 ± 0.8 in HS group (NS). In contrast to the control and HS groups, the number of positively stained cells for AngII in cortical interstitium was markedly elevated (9.5 ± 1.6 in the NAME group; *p < 0.05 versus control and HS).

AT₁ Receptor
The expression of AT₁ receptor was observed in all structures of the kidney: glomeruli, proximal, distal, and collecting tubules, and vessels, and in the interstitium. In animals with chronic renal disease, there was a large number of interstitial cells staining for AT₁ receptor, particularly in areas of severe renal damage (Figure 2F). The number of AT₁ receptor–positive cells in cortical interstitium was significantly increased in the NAME group (54.5 ± 7.8 AT₁+ receptor cells/mm²) compared with both the control and HS groups (1.3 ± 0.4 and 0.3 ± 0.1 cells/mm², respectively; *p < 0.01).

Effect of Enalapril, Losartan, and MMF
Table 1 shows the results of SBP, albumin excretion, and histology of the control and HS animals, rats with chronic renal disease (NAME group), and the NAME rats receiving enalapril, losartan, and MMF. Fifteen days after induction of renal disease, all drugs lowered BP. Losartan was the most effective antihypertensive drug, and MMF, despite being primarily an immunosuppressive drug, also lowered SBP. Thirty days after induction of renal disease, no drug was effective in lowering BP.

Treatment with enalapril or losartan induced a reduction of urinary albumin excretion at 15 d of observation. MMF treatment promoted a slight, but not statistically significant, attenuation of albuminuria. At 30 d, all drugs were ineffective in lowering albumin excretion, although the level of urinary albumin excretion decreased in the NAME+LOS group.
The effect of enalapril, losartan, or MMF on the interstitial AngII expression is presented in Figure 5. Interstitial AngII expression is increased in the NAME group compared with the control and HS groups, and the addition of drugs (losartan, enalapril, and MMF) either diminished (MMF) or normalized (losartan, enalapril) the expression AngII$^+$ cells in kidney interstitium at 15 d. However, this effect was not evident after 30 d. The number of interstitial cells expressing AngII parallels the severity of renal disease, as measured by SBP, albumin excretion, and histologic parameters.

Considering the mechanisms that might be involved in intrarenal AngII production, the expression of cathepsin D was also analyzed. Treatment with enalapril, losartan, or MMF induced a marked decrease in cathepsin D expression (64.3 ± 5.4, 63.9 ± 2.4, and 75.7 ± 3.4 cells/mm$^2$, respectively) compared with the NAME group at 30 d (104.7 ± 3.7 cells/mm$^2$; $P < 0.001$).

**Discussion**

In the study presented here, the expression of RAS components in the kidney, particularly AngII expression, was evaluated in a model of progressive renal disease. Animals receiving an inhibitor of nitric oxide synthase developed chronic renal disease characterized by hypertension, albuminuria, and histologic damage, as previously shown (19–22).

The analysis of systemic RAS showed no difference in PRA and plasma AngII levels between the NAME and control groups, although intense vasoconstriction occurred with the blockade of nitric oxide synthesis (19). One possible explanation is that a high-salt diet, which shuts off renin release, may have counterbalanced the strong ischemic stimulus to renin secretion. This seemed to be the case because PRA in the HS group was very low. Systemic RAS has been described to be either activated or depressed in this model, but these results could be influenced by different combinations of L-NAME dose and salt content in the diet used in the different studies (26–28). It is of note that HS rats presented suppressed PRA but not AngII plasma levels. These apparent paradoxical results were also reported by other groups (29,30), with no clear explanation. The dissociation between PRA, which was suppressed, and plasma AngII suggests that under this condition,
plasma AngII levels are mainly dependent of tissue-derived AngI and/or AngII. However, this possibility warrants further investigation.

In contrast to systemic RAS, there is a clear activation of intrarenal RAS in this model. All components of RAS were detected in the kidney, and some of them were upregulated. In this context, a considerable increase of intrarenal production of angiotensinogen was observed in Western blot experiments in kidneys of animals with chronic renal disease (NAME rats). Therefore, the precursor of AngII is upregulated in a pathologic situation and may be involved in local generation of AngII. Intrarenal angiotensinogen augmentation was also reported in experimental hypertension induced by AngII infusion (31) and in a model of proteinuria caused by BSA overload (32).

Renin expression was detected almost exclusively in the afferent arterioles, either in the control and HS groups or in animals with chronic renal disease. However, previous studies have reported the expression of small amounts of renin in the proximal tubule and in connecting ducts (33,34). In the study presented here, the expression of renin was diminished in HS rats, and it was not increased in rats with renal disease, which is in accordance with the observed PRA values. Thus, it seems that PRA reflected renin expression in the juxtaglomerular apparatus.

An alternative route to AngI generation that might be of relevance contributing to the local AngII production was analyzed. As mentioned before, cathepsin D is a renin-like enzyme that catalyzes angiotensinogen breakdown to AngI. This enzyme was detected in the collecting tubules and its expression was upregulated in animals with chronic renal disease. It should be stressed that cathepsin D operates at a low pH, which is coincident with the acidic condition found in the lumen of cortical collecting ducts. The recognition that angiotensinogen reaches the most distal segments of the nephron, as demonstrated by Kobori et al. (35), supports a possible role of a functionally active cathepsin D generating AngII in this part of the nephron. The normalization of cathepsin D expression in response to the administration of enalapril, losartan, or MMF strengthens the possibility that this enzyme is functionally active in local formation of AngII in the kidney.

In the study presented here, ACE was found in the brush border membrane of all segments of the proximal tubule. Similar results were reported by Metzger et al. (36), although ACE expression detected by these investigators was detected exclusively in the pars recta of rodent proximal tubules. The Western blot analysis demonstrated no difference in ACE protein content among the control, HS, and NAME groups. Moreover, the experiments analyzing ACE activity demonstrated that for the same content of ACE protein, the activity of ACE was unchanged (C-domain, Hippuryl-L-His-Leu substrate) or even depressed (N-domain, Z-Phe-His-Leu). It is well known that the AngI substrate is hydrolyzed by both sites of the ACE, although the two active sites (N- and C-domain) may cleave some substrates at different rates (37).

The results of the study presented here suggest that the ACE N-domain activity may be modulated in the presence of L-NAME plus a high-salt diet. Kashiwagi et al. (38) reported that the Hippuryl-L-His-Leu ACE activity was higher in rats receiving L-NAME. However, the activity of the N-domain was not analyzed, and in addition, the animals of this study did not receive a high-salt diet. To our knowledge, the effects of the summation of high salt intake and L-NAME on renal ACE activity have not been described until now, although it has been described that very low salt diet enhances kidney ACE activity in normal rats (39).

The definite evidence of local RAS activity in the intimacy of renal tissue is given by the presence of intrarenal AngII. In this study, we observed a marked increased expression of AngII in interstitial cells. Accordingly, augmentation of AngII renal tissue levels in this model has already been shown (38). The interstitial AngII may have a local action and the demonstration of an augmented expression of AT1 receptor in cortical interstitium seems to corroborate this local action, because both agonist and receptor are overexpressed in renal interstitium.

It is still unclear whether these positive cells reflect local production of AngII or eventually uptake of AngII produced in other sites of the kidney. There is a possibility that interstitial cells expressing AngII are actually producing the peptide intracellularly. In fact, renal fibroblasts in culture produce AngII, and this production is increased when these cells are stimulated with proinflammatory cytokines (40). However, in kidney specimens of our study, neither angiotensinogen nor ACE was found in interstitial cells. The lack of the detection of all components of the machinery required for AngII production by interstitial cells could be the result of a limitation of the immunohistochemical technique, which might have been unable to detect small amounts of those components. On the other hand, the observation that losartan diminished the number of interstitial cells expressing AngII raises the possibility that these cells are actually taking up AngII. However, other cells known to express AT1 receptor, like proximal tubule cells and vascular smooth muscle cells, which would be expected to be positive, do not express AngII. Thus, it remains unclear why AngII was not detected in other cells expressing AT1 receptor. One possible explanation is that the presence of AT1 receptor may be necessary but insufficient for intracellular AngII accumulation.

The analysis of the effect of different drugs (enalapril, losartan, and MMF) in this model demonstrated that all three drugs were renoprotective, as measured by BP, urinary albumin excretion, and histologic damage, at 15 d of treatment, but not after 30 d of treatment. The failure of these treatments in the late stage of the injury process may be the result of the high doses of L-NAME and salt content in the diet we used, leading to severe renal damage. Previous studies have already pointed to a relationship between L-NAME and salt doses with renal damage and response to RAS blockade (19,21,41). The option for a more aggressive model in this study aimed to detect differences in the intrarenal RAS expression, which might not be found in a more moderate model. Another possibility to explain the failure of the treatments at 30 d could be the conventional doses used in this study, which might have been...
suboptimal to confer renoprotection. Finally, the failure of RAS inhibitors to completely prevent the progression of renal disease in this animal model is somehow in accordance to the clinical observations, where blocking RAS does not completely arrest the progression of renal disease toward end-stage renal disease (42,43).

The number of AngII-positive interstitial cells seems to be associated to the degree of renal injury. It is also of note that enalapril, losartan, and MMF diminished renal AngII interstitial cells in this model at 15 d, but not at 30 d. It is still not clear whether AngII detected in the interstitial cells is totally regulated by ACE. In the same line of investigation, previous studies demonstrated that interstitial concentrations of AngII were not responsive to ACE inhibition, suggesting an independent regulation of renal interstitial AngII (14). This independent regulation may explain our findings of increased AngII in interstitial cells with lower ACE activity in NAME rats. It is possible that other alternative enzymatic pathways, such as chymases, may play a role in this system.

The specific actions of locally generated AngII are not yet elucidated. However, the detection of AngII in inflammatory cells is likely to be more related to local inflammation and fibrosis than to fluid volume regulation, suggesting a role of AngII as a “cytokine like molecule.” Accordingly, Antonipillai (44) has shown that inflammatory mediators such as TNF-α and IL-1β can induce renin release in renal tissue slices. In addition, TNF-α and IL-1β stimulate angiotensinogen production in the liver (45), and exposure of rats to LPS increases kidney angiotensinogen (46). Finally, AngII production is also enhanced in renal fibroblasts stimulated with IL-1β (40). Thus, it seems reasonable to assume that AngII could be locally produced in response to local inflammation.

In this setting, antiinflammatory drugs such as MMF have an important role in reducing inflammation and exerting its anti-proliferative effect, particularly in inflammatory cells. MMF may influence the intrarenal AngII generation not only by blocking the inflammatory signal but also diminishing the infiltration of cells that otherwise could be contributing to local AngII generation (22). The failure of this treatment observed at 30 d suggest that monotherapy with MMF was not sufficient to completely block the complex inflammatory process in this model.

In conclusion, this study demonstrated the presence of all components of RAS in the kidney, some of them being up-regulated locally in the kidney in a model of chronic progressive nephropathy. Additionally, the use of drugs that induced a decrease in the local AngII production ameliorated renal damage. Thus, local AngII production is probably involved in the pathogenesis of renal damage in this model of chronic kidney disease. [Printer: Reference (43) is cited here for parsing. Please delete this bracketed information.]

Acknowledgments

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References


