Role of Angiotensin II in Renal Injury of Deoxycorticosterone Acetate–Salt Hypertensive Rats

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Abstract To investigate the role of angiotensin II (Ang II) in hypertension-induced tissue injury, we gave TCV-116 (1 mg/kg per day PO), a nonpeptide Ang II type I receptor antagonist, or enalapril (10 mg/kg per day PO) to deoxycorticosterone acetate (DOCA)–salt hypertensive rats for 3 weeks and examined the effects on tissue mRNA levels for transforming growth factor-β1 (TGF-β1) and extracellular matrix components. Tissue mRNA levels were measured by Northern blot analysis. Renal mRNA levels for TGF-β1; types I, III, and IV collagen; and fibronectin in DOCA-salt hypertensive rats were increased by severalfold (P<.01) compared with sham-operated rats. In the aorta of DOCA-salt hypertensive rats, TGF-β1 and fibronectin mRNA levels were increased, but types I, III, and IV collagen mRNAs did not increase. In the heart, increased mRNA was found only for fibronectin. Thus, these gene expressions are regulated in a tissue-specific manner. TCV-116 or enalapril did not lower blood pressure in DOCA-salt hypertensive rats. However, the increase in renal mRNAs for TGF-β1 and extracellular matrix components in DOCA-salt hypertensive rats was significantly inhibited by treatment with TCV-116 or enalapril, which was associated with a significant decrease in urinary protein and albumin excretions and histological improvement of renal lesions. In contrast, in the aorta and heart these gene expressions were not affected by TCV-116 or enalapril. Thus, local Ang II may contribute to renal injury of DOCA-salt hypertension by stimulating the gene expression of TGF-β1 and extracellular matrix components. (Hypertension. 1994;24:195-204.)

Key Words • receptors, angiotensin • gene expression • transforming growth factor beta • extracellular matrix

The mechanism of development of hypertension-mediated renal injury remains to be determined, although hypertensive nephrosclerosis is one of the most important causes of end-stage renal failure.1–3 Investigations on the effect of angiotensin-converting enzyme (ACE) inhibitors4–8 or angiotensin II (Ang II) receptor antagonists9–11 on various renal diseases in humans and animals indicate that the renin-angiotensin system (RAS) participates in progressive renal injury as well as hypertension. Hemodynamic effects, such as the reduction of intraglomerular pressure by inhibition of the RAS, have been shown to contribute to a renal protective effect.4,5,10 However, recent studies have shown that the mechanism of renal protection by ACE inhibitors can also be partly attributed to unknown mechanisms other than the hemodynamic effect.12,13 Furthermore, accumulating evidence shows that a local RAS exists in the kidney and supports the notion that an intrarenal RAS, independent of the circulating system, may be involved in renal pathophysiology in a paracrine or autocrine fashion.14,15 However, the possible role of this local system in renal injury is poorly understood.

In recent years transforming growth factor-β1 (TGF-β1) has been shown to play an important role in tissue injury, including several experimental renal diseases, via stimulation of gene expression of extracellular matrix components.16–22 Extracellular matrix components in the interstitium mainly consist of types I and III collagen and fibronectin, although type IV collagen and laminin are the main extracellular matrix components present in the basement membrane. These proteins are implicated in cell adhesion, migration, and proliferation, thereby contributing to tissue injury.17,18,22 However, the gene expression of TGF-β1 and extracellular matrix components remains to be determined in hypertension-mediated renal injury. Furthermore, the in vivo effect of antihypertensive drugs on these gene expressions in tissue is also unknown.

To investigate the role of the local RAS in renal injury, we studied the effects of Ang II blockade on gene expression of TGF-β1 and extracellular matrix components in the kidney of deoxycorticosterone acetate (DOCA)–salt hypertensive rats and compared them with the effects in the aorta and heart. We obtained evidence that an intrarenal RAS is involved in the development of renal damage in DOCA-salt hypertensive rats by stimulating the gene expression of TGF-β1 and extracellular matrix components.
Methods

Drugs and Animals

TCV-116 and enalapril maleate were synthesized by Takeda Chemical Industries, Ltd. TCV-116 is a highly potent and selective nonpeptide angiotensin type 1 (AT1) receptor antagonist without agonistic action and has a long-acting antagonistic activity in vivo, thereby being a useful agent for examining in vivo the role of the AT1 receptor. DOCA was purchased from Wako Pure Chemicals and used as a 25-mg pellet form in our laboratories.

Five- to 6-week-old male Wistar rats (Wistar/Jcl, Clea Japan) weighing 150 to 170 g were used.

Experimental Protocol

All procedures were in accordance with institutional guidelines for animal research. Rats were anesthetized by injection of sodium pentobarbital (50 mg/kg IP). A left dorsal incision was made, the left kidney was removed, and a 25-mg DOCA pellet was subcutaneously implanted into the dorsum. The rats were fed a standard laboratory chow (CE-2, Clea Japan) and given 1% NaCl solution in the drinking water ad libitum.

Experimental Protocol

Sham-operated rats served as controls and were maintained on the same rat chow and given tap water ad libitum without the 1% NaCl solution. Three weeks after the operation, systolic blood pressure was measured by the tail-cuff method, and rats whose blood pressure was more than 180 mm Hg were used as DOCA-salt hypertensive rats. DOCA-salt hypertensive rats were divided into three groups based on their blood pressures and body weights and given vehicle, TCV-116, or enalapril for 3 weeks. There were no significant differences in body weights and blood pressures among the three groups before drug treatment was started. TCV-116 and enalapril were suspended with a small amount of gum arabic for oral administration. TCV-116 (1 mg/kg per day), enalapril (10 mg/kg per day), or vehicle (5% gum arabic solution) in a 0.83% Hypertension Vol 24, No 2 August 1994

Sham-operated rats were decapitated, trunk blood was collected from the tail artery of conscious rats for measurement of plasma renin concentration (PRC). After 3 days of blood collection, the cardiac left ventricle was separated from the right ventricle according to the method of Chomczynski and Sacchi with a minor modification. In brief, frozen tissues were homogenized with a polytron homogenizer (PCU-11, Kinematica AG) for 60 seconds at dial speed 10 in denaturing solution (4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate [pH 7.0], 0.1 mol/L 2-mercaptoethanol, and 0.5% N-lauroyl sarcosine). The homogenate was added to 1/10 vol of 2 mol/L sodium acetate (pH 4), 1 mol of water-saturated phenol, and 1/10 vol of chloroform and centrifuged at 10,000g at 4°C for 20 minutes. The resulting upper aqueous phase was transferred to a fresh tube and precipitated by addition of 1 vol of isopropanol followed by centrifugation. The precipitate was dissolved in the above denaturing solution, incubated with 3.5 mol/L lithium chloride at 4°C for 18 hours, and then centrifuged. After centrifugation the RNA pellet was washed with 3 mol/L lithium chloride and then with 70% ethanol. Finally, the RNA pellet was dissolved in 0.1% diethyl pyrocarbonate-treated water and stored at -80°C until use. RNA concentration was spectrophotometrically determined at 260 nm.

Northern Blot Hybridization

Twenty micrograms of total RNA from the renal cortex, aorta, and left ventricle were denatured by incubation with 1 mol/L deionized glyoxal and 50% dimethyl sulfoxide at 50°C for 1 hour as described26 and electrophoresed on a 1.1% agarose gel at 50 V. The 28S and 18S ribosomal RNAs in gels were stained with ethidium bromide to demonstrate the integrity of applied RNA and to verify that the same amounts of RNA were used in each lane. RNAs in the gel were then transferred to a nylon membrane (GeneScreen Plus, El du Pont de Nemours & Co, NEN Products). Each cDNA probe was labeled with [32P]dCTP (specific activity, 3000 Ci per millimoles per liter, Du Pont) by the random primer extension method27 with a Random Primer DNA Labeling Kit (Takara). Prehybridization and hybridization were performed according to the manufacturer's instructions. In brief, the membrane was prehybridized in a solution containing 50% formamide, 5 × Denhardt's solution (Ficoll, polyvinylpyrrolidone, and bovine serum albumin, 1 mg/mL each), 5 × SSPE (0.75 mol/L sodium chloride, 50 mmol/L sodium phosphate, 5 mmol/L EDTA acid), 1% sodium dodecyl sulfate, and 200 μg/mL denatured salmon sperm DNA at 42°C for 4 hours. Then the membrane was hybridized with [32P]-labeled cDNA (1 to 2 × 105 disintegrations per minute per milliliter) at 42°C for 24 hours in fresh hybridization solution that was identical to the prehybridization solution except for the absence of salmon sperm DNA. After washing, the membrane was exposed to Kodak XAR-5 film between two intensifying screens at -70°C. To strip off the hybridized probe, the nylon membranes were boiled in 0.1× SSC solution containing 1% sodium dodecyl sulfate for 30 minutes and then rehybridized with other cDNA probes. To evaluate tissue mRNA levels, we used an optical scanner (EPSON GT-8000, Seoko) for digitizing autoradiograms. The autoradiogram bands that were visualized with a digitizing image were measured for their density with the use of the public domain National Institutes of Health image program and a computer (Macintosh LC-III, Apple Computers, Inc). For all RNA samples, the density of an individual mRNA band was divided by that of the GAPDH mRNA band to correct for the difference in RNA loading and/or transfer.

cDNA Probes

cDNA probes were used as follows. Rat renin DNA was a 0.70-kb Kpn I–Kpn I fragment from Dr A. Fukamizu.28 Rat TGF-β1 cDNA was a 1.0-kb HindIII–Xba I fragment from Dr S.W. Qian.29 Rat fibronectin cDNA was a 2.07-kb HindIII– EcoRI fragment from Dr R.O. Hynes.30 Rat α1 (I) collagen cDNA was a 1.3-kb Pst I–BamHI fragment from Dr D. Rowe.31 Mouse cDNAs for α1 (III) collagen,22 α1 (IV) collagen,23 and laminin B1 chain24 were fragments of 1.8-kb EcoRI–EcoRI, 0.83-kb Ava I–Pst I, and 0.85-kb BamHI–EcoRI, respectively, from Dr Y. Yamada. Rat GAPDH was a 1.3-kb Pst I–Pst I fragment from Dr P. Fort.32

Measurement of Blood Pressure and PRC

Systolic blood pressure of conscious rats was measured by the tail-cuff method (Riken Kaibatsu PS-8000). PRC was measured as the rate of Ang I generation from rat plasma angiotensinogen, as described.24 In brief, rat plasma samples were incubated with nephrectomized rat plasma, and the generated Ang I was measured by a specific radioimmunoassay.
Histological Examination

DOCA-salt hypertensive rats, subjected to oral administration of vehicle or TCV-116 (1 mg/kg per day) for 3 weeks (from 3 to 6 weeks after operation) as described above, and sham-operated rats were decapitated, and the kidneys were removed, fixed in 10% phosphate-buffered formalin, embedded in paraffin, and cut into 4-μm-thick sections, which were stained with Azan for examination of renal fibrosis.

Miscellaneous Measurements

Urinary protein and albumin were measured with an A/G-B test and an Albumin-B test, respectively (Wako).

Statistical Analysis

All values are expressed as mean±SEM. Statistical significance was determined with ANOVA and Duncan's multiple range test. Differences were considered statistically significant at a value of P<.05.

Results

Effect of TCV-116 and Enalapril on Blood Pressure and Heart Rate

Two days before the start of drug administration, blood pressure in DOCA-salt hypertensive rats (3 weeks after operation) was significantly higher than in sham-operated rats (P<.01), and there was no significant difference in blood pressure among the three groups of DOCA-salt hypertensive rats before drug treatment (sham-operated rats and vehicle-treated, TCV-116–treated, and enalapril-treated DOCA-salt hypertensive rats, respectively, 131±2, 205±6, 201±5, and 196±6 mm Hg). On day 1 after the start of drug treatment, blood pressure of sham-operated rats and vehicle-treated, TCV-116–treated, and enalapril-treated DOCA-salt hypertensive rats was 142±5, 206±10, 195±6, and 197±9 mm Hg, respectively; on day 7, 124±5, 209±12, 202±6, and 197±5 mm Hg; on day 12, 123±4, 210±6, 209±5, and 202±5 mm Hg; and on day 18, 126±2, 215±8, 214±7, and 212±5 mm Hg (Fig 1A). Thus, TCV-116 and enalapril did not affect blood pressure of DOCA-salt hypertensive rats throughout 3 weeks of treatment.

There was no significant difference in heart rate among sham-operated rats and the three groups of DOCA-salt hypertensive rats 2 days before and on days 1, 7, 12, and 18 (Fig 1B) after the start of drug treatment.

Effect of TCV-116 and Enalapril on Body Weight and Renal, Cardiac, and Aortic Weights

Body weight before the start of drug treatment (3 weeks after operation) was not significantly different among sham-operated rats and the three groups of DOCA-salt hypertensive rats (data not shown). As shown in the Table, the body weights of DOCA-salt hypertensive rats after 3 weeks of vehicle treatment were smaller than those of sham-operated rats (P<.01). Three weeks of TCV-116 treatment significantly increased the body weights of DOCA-salt hypertensive rats (P<.05), but treatment with enalapril did not. In DOCA-salt hypertensive rats treated with vehicle, the weights of kidney, cardiac left ventricle, and aorta were all larger than in sham-operated rats (P<.01). Neither TCV-116 nor enalapril altered the weights of kidney, heart, or aorta in DOCA-salt hypertensive rats.

Effect of TCV-116 and Enalapril on Urinary Protein and Albumin Excretions

As shown in Fig 2A and 2B, urinary protein and albumin excretions (120.9±30.3 and 52.9±11.8 mg/24 h per 100 g body weight, respectively) in vehicle-treated DOCA-salt hypertensive rats were 3.7- and 20.3-fold larger, respectively, than those in sham-operated rats. Three weeks of treatment with TCV-116 or enalapril significantly decreased both urinary protein excretion (P<.05) and albumin excretion (P<.01), compared with vehicle-treated DOCA-salt hypertensive rats.

Effect of TCV-116 and Enalapril on PRC and Renal Renin mRNA

PRC on the first day of drug administration (3 weeks after operation) was 4.03±0.36, 1.10±0.12, 0.747±0.056, and 0.906±0.147 ng/L per second for sham-operated rats and vehicle-treated, TCV-116–treated, and enalapril-treated DOCA-salt hypertensive rats, respectively. Thus, on day 1 during dosing (3 weeks after operation), PRC in DOCA-salt hypertensive rats was significantly lower than in sham-operated rats (P<.01), and there was no significant difference in PRC among the three groups of DOCA-salt hypertensive rats. Fig 3A and 3B show PRC and renal renin mRNA levels, respectively, in sham-operated and DOCA-salt hypertensive rats at 3 weeks after the start of dosing (6 weeks after operation). There was no significant difference in PRC (2.98±0.76 versus 2.38±0.31 ng/L per second) and renal renin mRNA levels between vehicle-
Body Weight and Renal, Cardiac, and Aortic Weights in Sham-Operated and DOCA-Salt Hypertensive Rats After 3 Weeks of Treatment With Vehicle, TCV-116, or Enalapril

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Body Weight, g</th>
<th>Kidney Weight, mg/g body wt</th>
<th>Left Ventricular Weight, mg/g body wt</th>
<th>Aortic Weight, mg/g body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>336±8*</td>
<td>3.33±0.06*</td>
<td>2.00±0.03*</td>
<td>0.183±0.004*</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Vehicle</td>
<td>279±18</td>
<td>8.39±0.45</td>
<td>3.67±0.20</td>
<td>0.362±0.041</td>
</tr>
<tr>
<td>+TCV-116</td>
<td>318±5t</td>
<td>8.50±0.29</td>
<td>3.31±0.09</td>
<td>0.321±0.037</td>
</tr>
<tr>
<td>+Enalapril</td>
<td>305±11</td>
<td>8.79±0.47</td>
<td>3.39±0.10</td>
<td>0.376±0.033</td>
</tr>
</tbody>
</table>

DOCA indicates deoxycorticosterone acetate. Values are mean±SEM. Each group included six animals.

†P<.05, *P<.01 vs DOCA-salt + vehicle.

Effects of TCV-116 and Enalapril on Renal Cortical mRNA Levels for TGF-β1 and Extracellular Matrix Components in the Interstitium and Baseline Membrane

There was no significant difference in renal GAPDH mRNA values among sham-operated rats and DOCA-salt hypertensive rats treated with vehicle, TCV-116, or enalapril for 3 weeks. However, in the present study all mRNA values for TGF-β1 and extracellular matrix components were corrected for GAPDH mRNA values to confirm that the changes in mRNAs for TGF-β1 and extracellular matrix components were specific events.

As shown in Figs 4 and 5, renal cortical TGF-β1 mRNA levels in vehicle-treated DOCA-salt hypertensive rats were 2.4-fold higher than in sham-operated rats (P<.01). Both TCV-116 and enalapril significantly decreased renal TGF-β1 mRNA levels in DOCA-salt hypertensive rats to a similar extent (P<.01).

As shown in Figs 4 and 6, renal cortical mRNA levels for types I and III collagen and fibronecin in vehicle-treated DOCA-salt hypertensive rats were 3.1-, 4.6-, and 3.9-fold higher, respectively, than in sham-operated rats (P<.01). The renal cortical mRNA levels for these three extracellular matrix components in DOCA-salt hypertensive rats were significantly decreased by 3 weeks of treatment with TCV-116 or enalapril (P<.01).

![Graphs showing urinary protein excretion (Uprot V) and urinary albumin excretion (Ualb V) for sham-operated and DOCA-salt hypertensive rats treated with vehicle, TCV-116, or enalapril for 3 weeks of drug treatment.](image)

Fig 2. Bar graphs show urinary protein excretion (Uprot V) (A) and urinary albumin excretion (Ualb V) (B) in sham-operated and deoxycorticosterone acetate (DOCA)-salt hypertensive rats after 3 weeks of drug treatment. Abbreviations are as in Fig 1. Each bar represents mean±SEM; n=6 in each group.

![Graphs showing plasma renin concentration (PRC) and renal renin mRNA levels for sham-operated and DOCA-salt hypertensive rats treated with vehicle, TCV-116, or enalapril for 3 weeks of drug treatment.](image)

Fig 3. Bar graphs show plasma renin concentration (PRC) (A) and renal renin mRNA levels (B) in sham-operated and deoxycorticosterone acetate (DOCA)-salt hypertensive rats after 3 weeks of drug treatment. The ordinate in B shows the value of renal renin mRNA corrected for that of GAPDH mRNA; the mean value in the vehicle group is represented as 100. Abbreviations are as in Fig 1. Each bar represents mean±SEM; n=6 in each group.
for type I collagen, and \( P<.05 \) for type III collagen and fibronectin). There was no significant difference in these effects between TCV-116 and enalapril.

Figs 4 and 7 show renal cortical mRNA levels for basement membrane components. Type IV collagen mRNA levels in vehicle-treated DOCA-salt hypertensive rats were 3.0-fold higher than in sham-operated rats \( (P<.01) \) and were significantly decreased by treatment with TCV-116 or enalapril \( (P<.01) \). Renal cortical laminin B1 chain mRNA levels in vehicle-treated DOCA-salt hypertensive rats were 1.6-fold higher than in sham-operated rats \( (P<.05) \), and both TCV-116 and enalapril tended to decrease this mRNA \( (P=NS) \).

**Histological Findings in the Kidney**

Fig 8 shows photomicrographs of renal cortex. In vehicle-treated DOCA-salt hypertensive rats \( (n=14) \), the renal tubules showed atrophy of the epithelium, hypertrophy of the basement membrane, hyaline cast formation, and dilatation. Fibrinoid necrosis and atrophy of the capillary tufts were observed in the glomeruli. The arterioles showed prominent hypertrophy and fibrinoid necrosis. Fibrosis was observed in the interstitium. Three weeks of treatment with TCV-116 in DOCA-salt hypertensive rats \( (n=14) \) decreased atrophy of the epithelium, hypertrophy of the basement membrane, and hyaline cast formation in the renal tubules and also decreased fibrinoid necrosis in the glomeruli. Furthermore, TCV-116 treatment decreased interstitial fibrosis.

**Effect of TCV-116 and Enalapril on Aortic and Cardiac mRNA Levels for TGF-\( \beta \), Types I and III Collagen, Fibronectin, and Type IV Collagen**

Fig 9 shows typical autoradiograms of mRNAs from the aorta and heart. In the aorta of vehicle-treated DOCA-salt hypertensive rats, TGF-\( \beta \) and fibronectin mRNA levels, corrected for GAPDH mRNA levels, were 1.7-fold \( (P<.01) \) and 6.6-fold \( (P<.01) \) higher,
respectively, than in sham-operated rats, and types I and III collagen mRNA levels, corrected for GAPDH mRNA levels, were significantly lower in DOCA-salt than in sham-operated rats ($P<.01$). There was no significant difference in aortic type IV collagen mRNA levels between sham-operated and DOCA-salt hypertensive rats. These mRNA levels in DOCA-salt hypertensive rats were not significantly changed by 3 weeks of treatment with TCV-116 or enalapril.

In the heart, fibronectin mRNA levels corrected for GAPDH mRNA were 3.7-fold higher in DOCA-salt hypertensive than in sham-operated rats ($P<.01$) (Fig 9). However, no significant difference was found between the two groups in TGF-β1 and types I, III, and IV collagen mRNA levels. Neither TCV-116 nor enalapril affected these mRNA levels in the heart.

**Discussion**

In the present study we examined the gene expression of TGF-β1 and extracellular matrix components in the kidney, heart, and aorta of DOCA-salt hypertensive rats and also examined the effect of an AT$_1$ receptor antagonist and ACE inhibitor on these gene expressions. We found that the regulation of these gene expressions in DOCA-salt hypertensive rats is tissue specific and that the gene expression of TGF-β1, types I and III collagen, fibronectin, and type IV collagen is enhanced in the kidney of DOCA-salt hypertensive rats. Blockade of the RAS with TCV-116 or enalapril, despite no decrease in blood pressure, significantly inhibited these gene expressions in the kidney of DOCA-salt hypertensive rats. Furthermore, this inhibitory effect was associated with a significant reduction of urinary protein and albumin excretions and the histological improvement of renal lesions. Therefore, the present study suggests that the enhanced gene expression of renal TGF-β1 and extracellular matrix proteins is probably responsible for the development of renal injury in DOCA-salt hypertension. Local Ang II may contribute to renal injury of
DOCA-salt hypertension by stimulating these gene expressions.

The mechanism of the antiproteinuric effect by ACE inhibition or AT1 receptor blockade has been attributed to changes in renal hemodynamics such as reduction of glomerular capillary pressure.4,10 On the other hand, Heeg et al15 have found that systemic Ang II infusion in proteinuric patients treated with long-term ACE inhibition did offset the renal hemodynamic effects of ACE inhibition, whereas Ang II infusion did not affect the antiproteinuric effect. It has also been reported that both the systemic and renal hemodynamic effects of ACE inhibitors are maximal within hours and remain stable thereafter, whereas the maximal antiproteinuric response to ACE inhibitors occurs several weeks after initiation of ACE inhibition, thereby showing the dissociation between the time course of ACE inhibitor–induced hemodynamic effects and urinary protein excretion.13 Furthermore, morphological study demonstrates that inhibition of the RAS significantly lessens renal and extrarenal vascular structural injury in rats with chronic renal failure independent of the depressor effect on systemic blood pressure or local glomerular pressure.8 Thus, the mechanism of the renal protective effect of RAS inhibition remains to be elucidated and appears to be at least in part due to unknown mechanisms other than the hemodynamic effects.

TGF-β1, a multifunctional growth factor, contributes not only to cell growth, cell differentiation, and immunosuppression but also to the synthesis of extracellular matrix proteins.16 Extracellular matrix components are responsible for cellular adherence, migration, and proliferation and therefore play an important role in renal tubulo-interstitial and glomerular fibrosis.17,18,22 Recent data show that renal TGF-β1 gene expression is enhanced in several renal disease models, including mesangial proliferative glomerulonephritis,19 crescentic glomerulonephritis,20 and obstructive nephropathy,21 and this increased TGF-β1 is responsible for the accumulation of extracellular matrix components. Despite the possible importance of TGF-β1 in several renal disease models,19,21 the gene expression and possible role of TGF-β1 in hypertension-induced renal injury remain to be determined. These findings lead us to investigate the gene expression of TGF-β1 and extracellular matrix proteins in DOCA-salt hypertensive rats.

DOCA-salt hypertensive rats have been used extensively as a useful model of human hypertension and characterized develop progressive renal injury. In the present study TCV-116 and enalapril failed to lower blood pressure in DOCA-salt hypertensive rats throughout treatment, which probably can be explained by no activation of the circulating RAS in DOCA-salt hypertensive rats. Thus, the mechanism of hypertension in DOCA-salt hypertensive rats is not mediated by the RAS. In contrast, both drugs significantly reduced urinary protein and albumin excretions. TCV-116 histologically improved renal lesions to a significant extent in DOCA-salt hypertensive rats. Thus, Ang II blockade, without lowering blood pressure, was capable of significantly preventing renal damage in DOCA-salt hypertensive rats. Of note are the observations that renal cortical mRNA levels for TGF-β1; types I, III, and IV collagen; fibronectin; and laminin were increased in DOCA-salt hypertensive rats. Furthermore, our present histological observations with Azan staining showed increased interstitial collagen fibers (composed of types I and III collagen) and thickening of tubular basement membrane (mainly composed of type IV collagen) in DOCA-salt hypertensive rats, thereby supporting the hypothesis that the increased expression of collagen mRNAs led to the increased collagen proteins. It is possible that the enhanced gene expression of TGF-β1 and extracellular matrix components in DOCA-salt hypertensive rats may be only due to compensatory renal hypertrophy caused by unilateral nephrectomy. However, previous work on the renal gene expression of extracellular matrix components after unilateral nephrectomy in rats has revealed that types I and III collagen mRNA levels in the remaining kidney are unchanged until 7 days after unilateral nephrectomy and that type IV collagen and laminin mRNAs are increased, with a peak at 12 hours after unilateral nephrectomy, but return to control levels by 7 days.37 Furthermore, we have also examined the gene expression of TGF-β1 and extracellular matrix components in the remaining kidney at 1 and 8 weeks after unilateral nephrectomy in rats and have obtained evidence that mRNA levels for TGF-β1, fibronectin, type IV collagen, and laminin do not increase at 1 or 8 weeks after unilateral nephrectomy and that types I and III collagen mRNA levels are unchanged at 1 week and increased only by approximately 1.5-fold at 8 weeks after unilateral nephrectomy (S.K. et al, unpublished observations, 1994). Thus, the increased renal gene expressions in DOCA-salt hypertensive rats cannot be sufficiently explained by compensatory renal hypertrophy after unilateral nephrectomy. Both DOCA treatment and unilateral nephrectomy seem to be essential for the enhancement of the gene expression.
of TGF-β1 and extracellular matrix components in this hypertensive model.

In the present study TCV-116 and enalapril treatment significantly decreased renal TGF-β1 mRNA levels, which was accompanied by significant decreases in types I, III, and IV collagen and fibronectin mRNAs. In vitro studies using cultured cells show that Ang II stimulates TGF-β1 gene expression. Therefore, the suppression of renal TGF-β1 expression by the above two drugs may be mediated by their direct actions. Furthermore, TGF-β1 potently stimulates the gene expression of extracellular matrix components. Thus, the decreased expression of renal extracellular matrix components by Ang II blockade may be mainly secondary to the inhibition of TGF-β1 expression.

Accumulating evidence indicates that the RAS exists not only in the blood circulation but also in various organs, including the kidney, heart, and aorta. It has been reported that Ang II concentrations in fluids from various compartments of rat kidney are significantly higher than those in rat plasma. These findings, taken together with evidence for widespread localization of renal AT1 receptor in blood vessels, juxtaglomerular cells, interstitial cells, tubular cells, and glomeruli, suggest that the intrarenal RAS may have various unique pathophysiological functions. However, it is unclear which system contributes more to progressive renal injury—the circulating or local RAS. Our present work supports the notion that a local RAS rather than a circulating system may play an important role in renal injury of DOCA-salt hypertension.

It has been well established that renin is the limiting factor for the generation of Ang II in the blood circulation, because the plasma concentration of renin is much less than that of angiotensinogen and ACE. On the other hand, the in case of a local RAS, it is unknown which component is the limiting factor for Ang II generation—angiotensinogen, renin, or ACE. In contrast to the blood circulation, the contents of renin in the kidney are greater than those of ACE and angiotensinogen. Guan et al. who examined circulating and renal Ang II levels in two-kidney, one clip hypertensive rats, have demonstrated that there is a dissociation between intrarenal and circulating Ang II levels or between intrarenal Ang II and circulating renin levels in this hypertensive model and that the enhanced Ang II levels in the kidney of this model are due to the increase in renal ACE. Campbell et al. who have measured plasma and renal angiotensinogen, renin, and angiotensin peptides in the rat after ACE inhibition, also have found a similar dissociation and suggested that angiotensinogen may be a major rate-limiting determinant of angiotensin levels in the kidney. These findings support the notion that the activity of the intrarenal RAS may be determined not only by renin levels but also by angiotensinogen and ACE levels. Thus, it is suggested that components other than renin may play an important role in the activity of the intrarenal RAS in DOCA-salt hypertensive rats.

In vivo inhibition of Ang II production by ACE inhibitors increases the biosynthesis and secretion of renal renin because Ang II has a negative feedback effect on renal renin secretion and expression. In the present study, TCV-116 significantly elevated PRC, as did enalapril, thereby indicating a major role of the AT1 receptor in negative feedback regulation of renal renin secretion. Interestingly, despite the increased plasma renin, renal renin mRNA levels were not altered by TCV-116 or enalapril. The reason for this dissociation may be explained by the fact that renal renin expression is suppressed in DOCA-salt rats. In addition, we have previously reported that differently glycosylated multiple forms of renin are released into the circulation from the kidney of rats and cleared from the circulation by the liver at a different plasma half-life and that treatment of rats with ACE inhibitors causes the preferential renal release of the renin form with a longer plasma half-life. Thus, the increased plasma renin in DOCA-salt hypertensive rats after inhibition of the RAS may be partially due to the modification of the glycosylation of renin.

Previous studies have addressed the gene expression of TGF-β1 or extracellular matrix components in the aorta or heart of DOCA-salt hypertensive rats. It has been well established that renin is the limiting factor for the generation of Ang II in the blood circulation, because the plasma concentration of renin is much less than that of angiotensinogen and ACE. On the other hand, the in case of a local RAS, it is unknown which component is the limiting factor for Ang II generation—angiotensinogen, renin, or ACE. In contrast to the blood circulation, the contents of renin in the kidney are greater than those of ACE and angiotensinogen. Guan et al. who examined circulating and renal Ang II levels in two-kidney, one clip hypertensive rats, have demonstrated that there is a dissociation between intrarenal and circulating Ang II levels or between intrarenal Ang II and circulating renin levels in this hypertensive model and that the enhanced Ang II levels in the kidney of this model are due to the increase in renal ACE. Campbell et al. who have measured plasma and renal angiotensinogen, renin, and angiotensin peptides in the rat after ACE inhibition, also have found a similar dissociation and suggested that angiotensinogen may be a major rate-limiting determinant of angiotensin levels in the kidney. These findings support the notion that the activity of the intrarenal RAS may be determined not only by renin levels but also by angiotensinogen and ACE levels. Thus, it is suggested that components other than renin may play an important role in the activity of the intrarenal RAS in DOCA-salt hypertensive rats.

In conclusion, we obtained evidence that the gene expression of renal TGF-β1 is increased in DOCA-salt hypertension.
hypertensive rats, which may contribute to the increased expression of renal extracellular matrix proteins. An intrarenal AT₂ receptor, independent of systemic blood pressure, seems to be involved in the increased TGF-β1 expression in DOCA-salt hypertensive rats. Thus, we propose that the intrarenal RAS may play an important role in nephrosclerosis in DOCA-salt hypertension.

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References


Role of angiotensin II in renal injury of deoxycorticosterone acetate-salt hypertensive rats.
S Kim, K Ohta, A Hamaguchi, T Omura, T Yukimura, K Miura, Y Inada, T Wada, Y Ishimura and F Chatani

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