Effects of Angiotensin Inhibitors on Renal Injury and Angiotensin Receptor Expression in Early Hypertensive Nephrosclerosis

Hideaki Nakaya, Hiroyuki Sasamura, Yudai Kitamura, Tetsuro Amemiya, Konosuke Konishi, Matsuhiko Hayashi, and Takao Saruta

Angiotensin converting enzyme inhibitors (ACEI) are known to inhibit the progression of established renal failure. The aim of this study was to compare the efficacy of an ACEI and an AT1 receptor antagonist (AT1R-Ant) in preventing the development of renal disease, at an early stage of hypertensive nephrosclerosis. SHRSP/Izm rats (n = 61) were treated from 10 wk until 22 wk with the ACEI delapril (40 mg/kg/d) or the AT1R-Ant candesartan cilexetil (1 mg/kg/d). Proteinuria, and structural/ultrastructural changes were assessed at 14 and 22 wk. Treatment with either agent resulted in reductions in blood pressure and cardiovascular hypertrophy. Neither proteinuria nor major renal histological changes were evident at 14 wk. At 22 wk, however, proteinuria accompanied by nephrosclerotic changes was seen in the untreated SHRSP/Izm. Treatment with either ACEI or AT1R-Ant resulted in similar reductions in proteinuria (untreated, 32.2 ± 7.4; delapril-treated, 5.5 ± 1.2; candesartan-treated, 3.9 ± 0.3 mg/100 g/d). Prominent sclerosis of small-to-medium sized renal arteries was seen in the untreated SHRSP/Izm at 22 wk, but was similarly attenuated by the ACEI and AT1R-Ant. The glomerular ultrastructure was comparable between the two groups. No significant changes in renal AT1a or AT1b receptor subtype mRNA expression were seen throughout the course of the study. In contrast, a decrease in AT2 receptor mRNA was seen in the drug-treated groups at 14 wk but not at 22 wk. These results suggest that both ACEI and AT1R-Ant have similar efficacy in attenuating the onset of renal injury in early hypertensive nephrosclerosis, and that treatment with either agent is associated with a transient decrease in AT2 receptor mRNA expression. (Hypertens Res 1999; 22: 303-312)

Key Words: angiotensin, receptor, nephrosclerosis

Several clinical studies and meta-analyses have suggested that angiotensin converting enzyme inhibitors (ACEI) can attenuate the progression of renal disease, and to a greater degree than was initially expected based on their antihypertensive actions (1, 2). This has led to an increase in the clinical use of ACEI for patients with hypertension and renal insufficiency, in order to impede the progression to end-stage renal failure requiring dialysis or renal transplantation. More recently, AT1 receptor antagonists (AT1R-Ant) which directly inhibit the binding of angiotensin II (Ang II) to the AT1 receptor, have been developed. Results from animal experiments suggest that AT1R-Ant may as effective as ACEI in impeding the progression of established renal disease (3-5).

A different question is whether AT1R-Ant and ACEI are equally effective in attenuating the onset of hypertensive complications, including nephropathy, at a stage when such complications have not yet become apparent. This information is important clinically, because the number of patients who have not yet developed nephropathy is large. However, it is relatively difficult to implement clinical studies to study this issue prospectively, for two main reasons. First, only a small fraction of hypertensive patients develop nephrosclerosis, and thus a large number of patients must be recruited to perform such a study. Second, a long observation period is necessary before results can be analyzed.

Because of these difficulties, we addressed this question using SHRSP/Izm as an animal model of benign hypertensive nephrosclerosis. In this study, the structural/ultrastructural changes in the rat kidneys were examined at early points in the course of the disease (14 wk and 22 wk), and the effects of the two treatments in attenuating the onset of nephropathy were compared.

The second aim of this study was to examine the effects of ACEI and AT1R-Ant on AT receptor subtype mRNA expression in the kidney. Currently, 3 subtypes of AT receptors (AT1a, AT1b, and AT2) have been demonstrated in the rat kidney. The renal AT1 receptors are involved in renal vaso-
constriction, in growth and extracellular matrix production in the mesangium and vasculature, and in the control of proximal tubular function (6). On the other hand, the AT2 receptors may be involved in renal arteriolar vasodilatation (7), NO generation (8), and control of natriuresis (9). Like other agonist/receptor systems (for example β-receptor agonists and cardiac β-receptors), AT receptor expression is thought to be regulated by Ang II through a mechanism referred to as down- or up-regulation (10). Conversely, treatment with angiotensin inhibitors could also cause changes in AT receptor mRNA expression. In this study, we therefore examined whether ACEI or AT1R-Ant treatment was associated with permanent changes in AT receptor subtype mRNA expression in the kidney.

Methods

Animal Treatments

Studies were conducted using 10-wk-old male SHRSP Izumo strain rats (SHRSP/Izm) and normotensive Wistar-Kyoto controls (WKY/Izm) maintained by the Disease Model Cooperative Research Association, Kyoto, Japan. All experiments were performed in accordance with the Animal Experimentation Guidelines of the Keio University School of Medicine. Rats were allowed free access to a standard rat chow (CE-2; Nippon Clea, Tokyo, Japan) containing sodium 0.26 g/100 g and potassium 1.06 g/100 g and tap water ad libitum. Rats were randomly divided into 4 groups (n = 13-17 per group). Rats in groups 1 and 2 were untreated WKY/Izm rats and SHRSP/Izm rats, respectively. Rats in groups 3 and 4 were SHRSP/Izm rats treated with the ACEI delapril (40 mg/kg/d in drinking water) or the AT1R-Ant candesartan cilexetil (1 mg/kg/d in rat chow) for 12 wk (from age 10 to 22 wk), respectively. Some of the rats in each group (n = 4-5 per group) were sacrificed at age 14 wk, and the remainder at age 22 wk.

Assays

Systolic blood pressure and heart rate of awake animals were measured by tail-cuff plethysmography using a Natsume KN-210 manometer. Twenty-four-hour urine collection was performed in metabolic cages, and urinary protein, albumin and creatinine concentrations were determined using an autoanalyzer. Urinary β2-microglobulin (BM) and N-acetyl-D-glucosaminidase (NAG) were measured by latex agglutination and colorimetry assay, respectively. Plasma renin activity was determined by RIA of angiotensin I formed by incubation of plasma for 1 h at 37°C (11).

Histological studies

Kidneys and thoracic aortae were fixed in 10% phosphate-buffered formalin, then embedded in paraffin blocks. Histologic sections from the rat kidneys were stained with PAS, and sections from aortae were stained with Azan. Slides were examined by light microscopy, and renal histopathological changes were scored as previously described by our laboratory (12, 13). Over 50 glomeruli were examined from each individual rat, and the number of glomeruli exhibiting focal or global ischemic or proliferative damage was enumerated and expressed as a percentage of the total number of glomeruli examined. Blood vessels were graded 0 to 4 for arteriolar sclerosis according to the severity of hyalinosis and thickening of the vascular wall. Tubulointerstitial changes, including interstitial inflammation and tubular atrophy, were assessed and graded 0-3 as follows: grade 1, involvement of <20% of the cortical interstitium; grade 2, 20-40% of the interstitium; and grade 3, involvement >40%. Samples for transmission electron microscopy were fixed in 2.5% glutaraldehyde and embedded in epoxy resin prior to examination. Histological findings were checked by an experienced nephropathologist.

RT-PCR-RFLP (reverse transcription-polymerase chain reaction-restriction fragment length polymorphism) analysis of AT1 and AT2 receptor subtypes

Total RNA was purified from the kidneys of each animal by the acid guanidine-phenol-chloroform method (14), and quantified by measurement of absorbance of 260 nm in a spectrophotometer. AT1 and AT2 receptor subtype mRNA were analyzed by RT-PCR-RFLP, as described previously (15). In brief, 1 µg total RNA was reverse transcribed in a reaction mixture containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2 mM MgCl2, 1 mM dNTP, 1 U RNase inhibitor, 2.5 µM (50 pmol) random hexamers and 2.5 U Moloney murine leukemia virus reverse transcriptase in a volume of 20 µl. The reverse transcribed product was amplified with AT1, AT2 or GAPDH sense and antisense primers in a reaction mixture containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2 mM MgCl2, 0.2 mM dNTP, 15 pmol of each primer, 5 µCi 32P-dCTP, and 2.5 U Taq polymerase using a Perkin-Elmer-Cetus thermal cycler for 25 cycles. AT1 sense and antisense primers were as follows: 5'-GGAAACAGCTTTG-TGGTG-3' and 5'-GCACAATCGCCATAATTATG-3' for AT1a DNA, and one fragment of length 606 by in the case of AT1b (15). Reaction products were resolved in a 1% agarose gel and stained with ethidium bromide. The size of the fragments was determined by comparison with a 1-kb ladder (8). Reaction products were purified from each individual rat, and the number of glomeruli exhibiting focal or global ischemic or proliferative damage was enumerated and expressed as a percentage of the total number of glomeruli examined. Blood vessels were graded 0 to 4 for arteriolar sclerosis according to the severity of hyalinosis and thickening of the vascular wall. Tubulointerstitial changes, including interstitial inflammation and tubular atrophy, were assessed and graded 0-3 as follows: grade 1, involvement of <20% of the cortical interstitium; grade 2, 20-40% of the interstitium; and grade 3, involvement >40%. Samples for transmission electron microscopy were fixed in 2.5% glutaraldehyde and embedded in epoxy resin prior to examination. Histological findings were checked by an experienced nephropathologist.
with a laser image analyzer (model BAS 2000; Fuji Film Co., Tokyo, Japan).

**Fig. 1. Effects of delapril and candesartan cilexetil on blood pressure in SHRSP/Izm.** Blood pressures in the treated groups were significantly reduced compared to those of untreated SHRSP/Izm at all time points after start of treatment (p<0.01, symbols omitted for clarity). Delapril: SHRSP/Izm treated with delapril (40 mg/kg/d). Candesartan: SHRSP/Izm treated with candesartan cilexetil (1 mg/kg/d).

**Table 1. Biochemical and Structural Parameters in SHRSP/Izm**

<table>
<thead>
<tr>
<th></th>
<th>WKY control n=5</th>
<th>SHRSP control n=5</th>
<th>SHRSP delapril n=4</th>
<th>SHRSP candesartan n=4</th>
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<tr>
<td>14 wk</td>
<td></td>
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<tr>
<td>Blood pressure (mmHg)</td>
<td>140±2**</td>
<td>216±6**</td>
<td>180±2**††</td>
<td>178±3**††</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/h)</td>
<td>2.5±0.5</td>
<td>2.5±0.6</td>
<td>5.7±0.5**††,ccc</td>
<td>10.7±0.2**††,dd</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Kidney weight (one side) (g)</td>
<td>0.82±0.07</td>
<td>0.88±0.05</td>
<td>0.84±0.05</td>
<td>0.80±0.03</td>
</tr>
<tr>
<td>Aortic wall thickness (media/lumen ratio ×100)</td>
<td>5.7±0.3††</td>
<td>7.8±0.2**</td>
<td>7.1±0.2**</td>
<td>7.0±0.3**</td>
</tr>
<tr>
<td>Heart weight/body weight (g/100 g)</td>
<td>0.7±0.08</td>
<td>0.64±0.04</td>
<td>0.78±0.06</td>
<td>0.74±0.06</td>
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<td>Heart weight (g)</td>
<td>0.81±0.07</td>
<td>0.8±0.03</td>
<td>0.92±0.09</td>
<td>0.93±0.07</td>
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<td>Body weight (g)</td>
<td>236±12</td>
<td>248±7</td>
<td>230±7</td>
<td>250±4</td>
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<tr>
<td>22 wk</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Blood pressure (mmHg)</td>
<td>135±1**</td>
<td>215±3**</td>
<td>165±4**††</td>
<td>164±3**††</td>
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<tr>
<td>Plasma renin activity (ng/ml/h)</td>
<td>2.3±0.7</td>
<td>7.8±1.0</td>
<td>11.0±2.4**</td>
<td>14.5±1.6**††</td>
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<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>0.44±0.02</td>
<td>0.52±0.03</td>
<td>0.44±0.03</td>
<td>0.44±0.02</td>
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<td>Creatinine clearance (ml/min)</td>
<td>1.33±0.1</td>
<td>0.88±0.1</td>
<td>1.07±0.2</td>
<td>1.51±0.5</td>
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<tr>
<td>Kidney weight (one side) (g)</td>
<td>1.36±0.02††</td>
<td>1.13±0.06**</td>
<td>1.11±0.02**</td>
<td>1.13±0.03**</td>
</tr>
<tr>
<td>Aortic wall thickness (media/lumen ratio ×100)</td>
<td>6.2±0.1††</td>
<td>8.1±0.2**</td>
<td>7.0±0.2††</td>
<td>6.7±0.3††</td>
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<tr>
<td>Heart weight/body weight (g/100 g)</td>
<td>0.33±0.01††</td>
<td>0.55±0.03**</td>
<td>0.45±0.01**</td>
<td>0.40±0.03**</td>
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<tr>
<td>Heart weight (g)</td>
<td>1.27±0.03</td>
<td>1.37±0.04</td>
<td>1.13±0.03††</td>
<td>1.06±0.04**</td>
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<tr>
<td>Body weight (g)</td>
<td>385±6</td>
<td>252±12**</td>
<td>256±7**</td>
<td>262±4**</td>
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<tr>
<td>N-acetyl-beta-D-glucosaminidase (U/d)</td>
<td>0.1±0.02</td>
<td>0.13±0.03</td>
<td>0.09±0.01</td>
<td>0.09±0.01</td>
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<tr>
<td>Beta 2-microglobulin (mg/d)</td>
<td>235.4±79.1</td>
<td>175.8±25.4</td>
<td>166.1±48.4</td>
<td>132.8±33.3</td>
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</table>

Results shown are the mean±SEM. *,**: p<0.05, p<0.01 vs. WKY, †,**†: p<0.05, p<0.01 vs. SHRSP, †,‡: p<0.01 vs. candesartan, †,‡: p<0.01 vs. delapril, ND: not determined.
Table 1 shows the effects of the treatments on cardiac and vascular hypertrophy, which were assessed by measurement of heart weight/body weight, and aortic lumen/media ratios, respectively.

Effects of delapril and candesartan cilexetil on serum and urine chemistries, and cardiovascular hypertrophy in 14-wk-old and 22-wk-old SHRSP/Izm. As shown in Table 1, treatment of SHRSP/Izm with delapril or candesartan caused increased plasma renin activity at both 14 and 22 wk. In contrast, plasma creatinine levels, 24-h creatinine clearance, urine NAG excretion, and urine β2-microglobulin excretion were not significantly different between treatment groups.

Table 1 also shows the effects of the treatments on cardiac and vascular hypertrophy, which were assessed by measurement of heart weight/body weight, and aortic lumen/media ratios, respectively.
Both cardiac and vascular hypertrophy were evident at 22 wk in the untreated SHRSP, but treatment with either delapril or candesartan resulted in significant attenuation of the cardiac and vascular changes.

Effects of delapril and candesartan cilexetil on proteinuria and albuminuria in 14-wk-old and 22-wk-old SHRSP/Izm
Fourteen-week-old SHRSP/Izm did not display any significant proteinuria or albuminuria when compared to WKY/Izm rats of the same age. In con-
In contrast, a 6-fold increase in urine protein excretion, together with comparable changes in urine albumin, was seen in the 22-wk-old SHRSP/Izm. Treatment with either delapril or candesartan resulted in reduction of the proteinuria to levels similar to those in the WKY/Izm rats (Fig. 2).

Fig. 6. Glomerular ultrastructure in 22-wk-old SHRSP/Izm rats. Representative photomicrographs of glutaraldehyde-fixed samples examined by transmission electron microscopy are shown. dela: SHRSP/Izm treated with delapril (40 mg/kg/d). cand: SHRSP/Izm treated with candesartan cilexetil (1 mg/kg/d). No major differences in the basement membrane (arrowheads) or podocyte structure (arrows) were seen.

Fig. 7. Representative image of RT-PCR-RFLP analysis of AT receptor subtype mRNA levels in the kidneys at 14 wk and 22 wk. Bands corresponding to AT1a and AT1b mRNA (upper panel), AT2 mRNA (middle panel) and GAPDH (lower panel) are shown. dela: SHRSP/Izm treated with delapril (40 mg/kg/d). cand: SHRSP/Izm treated with candesartan cilexetil (1 mg/kg/d).
Effects of delapril and candesartan cilexetil on renal histological changes in 14-wk-old and 22-wk-old SHRS/1zm

Representative photomicrographs of renal histology by light and electron microscopy in the various groups at 14 and 22 wk are shown in Figs. 3 and 4, and a summary of the histological scoring is shown in Fig. 5. At 14 wk, little mesangial expansion was noted in the glomeruli of SHRS/1zm. No vascular hypertrophy or interstitial changes were seen at this age. In contrast, marked hypertrophy of the small- to-medium sized arteries were seen in the kidneys of 22-wk-old SHRS/1zm, but not in WKY/1zm of the same age (Fig. 4). These vascular lesions were characterized by intimal and medial hyperplasia, with hyalinosis and/or thrombosis. Many of the glomeruli were intact in the SHRS/1zm, and were similar in appearance to glomeruli from WKY/1zm. However, other glomeruli displayed marked ischemic collapse and/or sclerosis. Tubular atrophy and interstitial inflammation were also seen. These histological changes (vascular, glomerular, and interstitial) were significantly reduced in both the delapril- and candesartan-treated groups (Fig. 5).

In order to confirm that there were no histological differences between the delapril- and candesartan-treated groups which could be missed by light microscopy, electron microscopy sections were also examined by a nephropathologist. Examination of the glomerular basement membrane, endothelial cells, and epithelial podocytes did not reveal ultrastructural changes between the treated groups (Fig. 6).

Effects of delapril and candesartan cilexetil on AT receptor subtype mRNA in 14-wk-old and 22-wk-old SHRS/1zm

Levels of AT receptor mRNA were examined using RT-PCR. In the case of the AT1a and AT1b receptors, no significant changes were seen between the treatment groups at 14 wk or 22 wk. In contrast, AT2 receptor mRNA was reduced in the treated groups at 14 wk, but returned to baseline at 22 wk, as shown in Figs. 7 and 8.

Discussion

Despite the development of new and improved class-
es of antihypertensive agents, hypertensive nephrosclerosis as a cause of end-stage renal disease is increasing every year in Japan and other countries. Two strategies can be proposed to reduce the incidence of this disease. One is to inhibit the progression of established renal failure. The other is to prevent the onset of hypertensive renal disease.

Concerning the former strategy, ACEI have been shown to reduce the progression of chronic renal failure in human studies (1, 2). This action may be related to reduction of the high intraglomerular pressures seen in advanced renal disease (19). The efficacy of AT1R-Ant as compared to that of ACEI in preventing the progression of renal disease has not yet been determined clinically, but has been studied in many animal models, including remnant kidney, DOCA-salt, immunological, and nephrotoxic models, as well as normal-salt and salt-loaded SHRSP (for review see Kim and Iwao (20), Tarif and Bakris (21)). To date, most of these studies have suggested that AT1R-Ant may be as efficacious as ACEI in inhibiting disease progression in these models.

Currently, the optimum therapeutic strategy for attenuating the onset of hypertensive renal disease in patients without established renal disease has not been established. As stated in the introduction, while this question is important clinically, the design of trials which might resolve this question is complex, and results will not be available for some time. Therefore, in this study, we examined the effects of treatment with the ACEI delapril and the AT1R-Ant candesartan on the early stages of nephropathy, using SHRSP/Izm on a regular salt diet as a model of benign hypertensive nephrosclerosis.

In order to recognize early signs of nephrosclerosis, renal changes were analyzed at 14 wk as well as at 22 wk. At 14 wk, no proteinuria was seen, and the renal histology appeared almost intact. In contrast, histological changes and proteinuria were evident in the untreated SHRSP/Izm at 22 wk. These changes were similarly attenuated in both the treated groups.

Because the changes seen in early hypertensive nephrosclerosis may be fine, glomerular ultrastructural changes were also examined by a nephropathologist in order to rule out minor differences between treatment groups. However, the glomerular morphology of the different groups appeared similar when examined by electron microscopy. Moreover, comparison of markers of renal tubular damage (NAG and β2-microglobulin) did not reveal any significant changes.

Theoretically, ACEI may be considered to differ from AT1R-Ant because of their ability to inhibit kininase II, which results in increased bradykinin production. On the other hand, selective inhibition of the AT1 receptor may have the benefit of attenuating actions of Ang II produced by ACE-independent pathways, and could maintain stimulation of AT2 receptors (6). However, the results of this study suggest that, despite the potential theoretical advantages and disadvantages of ACEI and AT1R-Ant, in practice the two appear to have virtually identical effects on the developing renal changes in SHRSP. This suggests that the above effects either cancel each other out, or are negligible compared to the major action of both these agents in attenuating stimulation of the AT1 receptor by Ang II. The effects of ACEI and AT1R-Ant on older SHRSP have been reported by other groups (4, 22). Taken together, these previous and our present results suggest that AT1R-Ant may be as effective as ACEI in preventing both the onset and the progression of hypertensive nephrosclerosis in this rat model.

In this study, we also examined the effects of ACEI and AT1R-Ant treatment on expression of renal AT receptor subtype mRNA. Two major classes of AT receptors are expressed in the rat kidney (6). The AT1 receptor is expressed in renal glomeruli, vasculature, and tubules, and is thought to mediate almost all the ‘classical’ actions of Ang II in the kidney, including control of renal vasconstriction and proximal tubule function. In rodents, there are two related AT1 receptor subtypes (AT1a and AT1b subtypes) with high homology (93%). Currently, these two receptors are thought to have virtually identical signal transduction mechanisms and biological actions.

The AT2 receptor, on the other hand, is highly expressed in fetal tissues, but the expression decreases dramatically after birth (6). In the adult kidney, expression of the AT2 receptor has been reported in the renal vasculature, particularly in such larger vessels as the interlobular arteries (23, 24), as well as in the glomeruli (25). AT2 receptor expression has also been detected in cultured glomerular endothelial and epithelial cells (26, 27).

Consistent with the reported distribution of the AT2 receptor in the kidney, several groups have suggested a role for AT2 receptor in the control of renal hemodynamics, diuresis, and natriuresis. Lo et al. reported that the AT2 agonist CGP123312A decreased natriuresis and urine flow in rats, whereas the AT2 antagonist PD 123319 had the opposite effect (9). Siragy et al. reported that the AT2 receptor may be involved in the control of nitric oxide and cGMP production (8). Recently Arima et al. reported that afferent arteriole activation of the AT2 receptor may cause endothelium-dependent vaso-dilation via a cytochrome P-450 pathway (7). Taken together, these data suggest that the AT2 receptor is involved in modulation of renal hemodynamics through interactions with local mediators.

Concerning the AT1 subtype, Ang II has been reported to cause downregulation of AT1 receptor mRNA in cultured vascular smooth muscle cells (28), although Iwai and Inagami found that Ang II did not affect AT1 receptor mRNA in the kidney or aorta when administered in vivo (29). Consistent with previous reports, we did not find a major effect of the angiotensin inhibitors on AT1 receptor mRNA in the kidney (22, 29) even when the drugs were administered for a total of 12 wk. Clinically, this suggests that there are few concerns regarding changes in renal AT1 receptor expression even with long-term treatments with ACEI or AT1R-Ant.
In contrast, we found a transient decrease in AT2 receptor mRNA after 4 wk, but not after 12 wk, of treatment. At present, few studies have addressed the question of AT2 receptor regulation in the kidney, so the reasons for the observed changes are unclear. The changes in AT2 receptor expression could be a consequence of the hypotensive effects of the angiotensin inhibitors. Although this possibility cannot be ruled out, it appears relatively unlikely, since no decrease in AT2 mRNA was seen in the normotensive WKY controls. Moreover, the fact that the downregulation was seen with both the ACEI and AT1R-Ant suggests that the changes were not mediated through the AT2 receptor itself (homologous regulation) but by an AT1-mediated mechanism (heterologous regulation).

Interestingly, Ozono et al. recently studied the effects of sodium depletion for 1 wk on expression of AT2 receptor protein in young adult (4-wk-old) and mature (3-mo-old) normotensive Sprague-Dawley rat kidneys. In both the young and mature rats, sodium depletion resulted in increased expression of AT2 receptors in the glomeruli and interstitial cells of the kidney (23). They did not study the effect of angiotensin inhibitors on the low sodium-induced upregulation. However, low sodium is known to cause activation of the renin-angiotensin system, so it is possible that Ang II may have been involved in the upregulation of renal AT2 receptors in their study.

Although the effects of Ang II on AT2 receptor regulation have not been studied in kidney cells in vitro, the effects on cells from other tissues have been studied by two groups. Shibata et al. reported that Ang II causes upregulation of AT2 receptors in rat cortical neurons by a protein kinase C-indepen dent, serine/threonine phosphatase-dependent mechanism (30). This effect was unchanged by the AT1 receptor antagonist SC-52458, but inhibited by the AT2 receptor antagonist PD123319. On the other hand, Kijima et al. reported that Ang II caused downregulation of AT2 receptor mRNA in myocytes by a protein kinase C-dependent mechanism (31). These different results suggest tissue-specificity in the mechanism of regulation of AT2 receptors by Ang II, and suggest that both AT1 and AT2 receptors may be involved in regulation of AT2 receptors in different tissues. Therefore further in vitro studies are required to clarify the mechanism of the changes in AT2 receptor mRNA seen in our study.

The physiological implications of the changes in AT2 receptor mRNA also requires further study. Because many of the actions of AT2 receptors in the kidney are antagonistic to the effects of AT2 receptor stimulation, we speculate that the reduction in AT2 receptor mRNA may be a compensatory response to limit the effects of AT1 inhibition.

In summary, our results suggest that both ACEI and AT1R-Ant appear to have similar nephroprotective effects at the start of early hypertensive nephrosclerosis in the rat. Treatment with either angiotensin inhibitor was associated with transient decreases in AT2 receptor mRNA. Further studies are required to examine the mechanisms of the changes in receptor regulation, as well as their physiological implications.

References
17. Shanmugam S, Lenkei ZG, Gasc JR, Corvol PL,


