Regulation of Angiotensin Type 1 Receptor and Its Gene Expression: Role in Renal Growth

DONNA H. WANG,* YONG DU,* HUAWEI ZHAO,* JOEY P. GRANGER,† ROBERT C. SPETH,‡ and DONALD J. DIPETTE*  
*Department of Internal Medicine, Hypertension and Vascular Research Laboratories, University of Texas Medical Branch, Galveston, Texas; †Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi; and ‡Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, Washington.

Abstract. Low sodium intake has been demonstrated to upregulate the predominant renal type 1 angiotensin II (Ang II) receptor (AT1), the AT1A subtype. The study presented here tests the hypothesis that the upregulation of renal AT1 mRNA induced by sodium depletion occurs conjointly with an elevation of the AT1 receptor that modulates renal growth. Seven-week-old male Wistar rats were divided into four groups and treated for 2 wk with normal sodium diet, normal sodium diet plus 3 mg/kg/day losartan, low sodium diet, or low sodium diet plus losartan. Body weight and MAP were not significantly different among the four groups. Plasma renin activity was significantly elevated by losartan treatment, low salt intake, or a combination of the two, compared with their levels in controls. Radioligand binding assays revealed that AT1 receptors were significantly increased by low salt intake but were significantly decreased by losartan treatment. Renal AT1 receptor binding in the rats subjected to sodium depletion plus losartan did not differ from that in control rats. Kidney weight, kidney weight/body weight ratio, and renal DNA and protein content were not altered by sodium depletion but were significantly lowered by losartan treatment with both normal and low sodium intake, compared with those of controls. The protein/DNA ratio was not significantly different among the four groups. Blockade of renal AT1 receptors with losartan was found to retard normal renal growth, indicating that Ang II is required for normal renal development. Low sodium intake was found to increase mRNA and expression of the renal AT1 receptor but to have no effect on renal growth, suggesting that an increase in renal mass above a normal level requires the activation of multiple factors. Blockade of the AT1 receptor by losartan was found to upregulate AT1 mRNA but to downregulate the AT1 receptor, suggesting that AT1 receptor-mediated intracellular events are necessary to sustain functional AT1 receptor expression in the kidney. (J Am Soc Nephrol 8: 193–198, 1997)

The renin-angiotensin system (RAS) plays an important role in blood pressure regulation, electrolyte balance, and extracellular fluid volume control. The effects of angiotensin II (Ang II) are mediated by binding to specific receptors. With the use of a competitive in vitro autoradiographic ligand binding method, it has been shown that the type 1 Ang II receptor (AT1) is the predominant renal subtype (1–3). Recently, we have demonstrated that gene expression of the AT1A receptor, which is the predominant AT1 subtype in the kidney is upregulated by low sodium intake (4), suggesting that expression of the AT1 receptor in the kidney is linked with salt and water homeostasis.

Interestingly, AT1 gene expression is also elevated in the kidney during fetal life compared with it expression in the adult animal, suggesting a role for Ang II in renal growth and maturation (5). In support of this concept, we have previously reported that the angiotensin converting enzyme (ACE) inhibitor, captopril, prevents the normal growth of blood vessels in the rat (6). In addition, administration of captopril to one-kidney, one-clip hypertensive rats reduces the wall mass of both the aorta and skeletal muscle arterioles (7). These growth-inhibitory effects of ACE inhibition are independent of concomitant hemodynamic changes (6,7).

Direct evidence for an effect of Ang II on normal renal growth was lacking until the recent report of Tufo-McReddie et al. (8). This study showed that the selective AT1 blocker, losartan, inhibits normal renal and somatic growth in rats. These results raised the intriguing question of whether activation of AT1 gene expression by low sodium intake induces abnormal renal growth. If the AT1 gene is linked with abnormal renal growth, knowledge of this link could provide further insight into the relevance of AT1 receptors in hypertensive and renal disease states. For example, it has been noted that compensatory renal growth may result in the development of glomerulosclerosis and interstitial fibrosis associated with irreversible loss of functioning nephrons (9), and the ultimate
outcome of loss of nephrons in the kidney is end-stage renal disease, e.g., renal failure. Moreover, clinical observations indicate that systemic hypertension is found in virtually all subjects with fewer than $0.8 \times 10^6$ glomeruli per kidney (10). Thus, determination of the mechanism(s) underlying renal growth is important. In the study presented here, we used Northern blot analysis combined with radioligand binding to test the hypothesis that increased AT1 gene expression by sodium restriction results in an increase in AT1 receptor density that consequently modulates renal growth.

**Materials and Methods**

**Treatment Groups**

Young 7-wk-old male Wistar rats weighing 179 ± 4 g (Charles River Laboratories, Inc., Wilmington, MA) were randomly divided into four groups ($N = 13$ in each group) and treated for 2 wk with normal sodium diet (0.5%), normal sodium diet plus losartan, low sodium diet (0.07%), or low sodium diet plus losartan. The rat food was purchased from Harlan Teklad Diets (Madison, WI). Losartan (3 mg/kg/day) was dissolved in 0.5 mL of water and given by oral gavage. This dose of losartan was chosen because it has previously been demonstrated to provide angiotensin II antagonism without lowering blood pressure (personal communication with Dr. Pancras C. Wong, DuPont Merck Pharmaceutical Company, Wilmington, DE). An equal amount of water was given by oral gavage to both the NS and LS rats. All animal procedures were in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

**Plasma Renin Activity**

At the end of the 2-wk diet treatments, all the rats were anesthetized with a single intraperitoneal injection of 100 mg/kg of thiopental sodium. The right carotid artery was catheterized for the measurement of mean arterial pressure (MAP) with a Statham 231 D pressure transducer (Gould Inc., Cleveland, OH) coupled to a Gould 2400S recorder (Gould Inc.). The rats were hepaminized (1 unit/g), and samples of plasma (3 mL) were collected from the carotid artery and placed in chilled ethylenediamine-tetraacetic acid (EDTA) tubes. Plasma renin activity (PRA) was determined using a commercially available radioimmunoassay kit for angiotensin I (Instar Co., Stillwater, MN).

**Tissue Preparation and Measurement of DNA and Protein Content**

To obtain tissue for Northern blot analysis, a midline incision in the abdomen was performed. The left kidney was promptly removed, and the right kidney was divided into three pieces, abdomen was performed. The left kidney was promptly removed, and the right kidney was divided into three pieces, and LS rats. All animal procedures were in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

**cDNA Probes**

The clone pUC19 containing a 2.3 kb of the rat AT1 receptor (a generous gift of Dr. Tadashi Inagami, Vanderbilt University School of Medicine, Nashville, TN) (14) was digested with Kpn I and EcoR I to obtain a 790-bp fragment (~180 to +610). This fragment was used as a template for making AT1 cDNA probes. The AT1 cDNA probes were labeled with $^{32}$P-dCTP using a Multiscribe DNA labeling system (Amersham Co., Arlington, IL) to a specific activity of $3 \times 10^8$ CPM/µg. The labeled probes were separated from uncoprecipitated nucleotide using Mini-Spin G-50 DNA purification spin columns (Worthington Biochemical Co., Freehold, NJ).

**RNA Extraction and Northern Blots**

Total RNA of kidney was extracted using the guanidine thiocyanate-phenol-chloroform extraction protocol (15). Electrophoresis of $30$ µg denatured RNA from each preparation was performed in a 1% agarose gel containing 2.2 mol/L formaldehyde. RNA was transferred to a positively charged nylon membrane (Fisher Co., Houston, TX). The membrane was baked at 80°C for 2 h in a vacuum oven (Fisher Co., Houston, TX) and prehybridized for 5 h at 42°C in hybridization buffer—50% deionized formamide, 5X Denhardt’s solution, 5X standard saline citrate (SSC), 0.5% sodium dodecyl sulfate (SDS), and 200 µg/mL of denatured salmon sperm DNA. The membrane was hybridized with the $^{32}$P-labeled probes in the hybridization buffer for 18–20 h at 42°C. Then it was washed successively in 2X, 1X, and 0.5 X SSC (two times, 10 min each) containing 0.1% SDS. Stringencies of washes were 65°C. Blots were exposed to XAR-5 X-ray film (Eastman Kodak Co., Rochester, NY) with intensifying screen. To correct the differences in RNA loading, Northern blots were incubated at 90°C for 10 min in 200 tris(hydroxymethyl)-aminomethane (Tris)-HCl (pH 8.0) to strip off the cDNA probes and rehybridized with $^{32}$P-labeled glyceraldehyde 3 phosphate dehydrogenase (GAPDH) cDNA probes. Autoradiographic signals were scanned with a laser densitometer (Ultrascan XL, Pharmacia). Relative gene expression was expressed as the ratio of AT1 mRNA to GAPDH mRNA.

**Ligand Binding Assay**

Tissues were homogenized in hypotonic buffer (20 mM sodium phosphate, pH 7.1–7.2). Homogenates were then centrifuged at 48,000 g for 20 min at 4°C. Cell membranes were resuspended in assay buffer (50 mM sodium phosphate, 150 mM NaCl, 1 mM EDTA, 0.1 mM bacitracin, pH 7.2) and recentrifuged. Bacitracin and EDTA are included in the assay buffer to inhibit peptidase activity in the tissue preparation. After resuspension in assay buffer, an aliquot of the cell membrane suspension was used for protein assay using modified Bradford dye-binding procedure (Bio-Rad, Hercules, CA) (13). To measure total Ang II receptors, $100$ µg protein was incubated with 62.5 p.m. to 2 nM mono-25I-(Sar, Ile) Ang II prepared as described by Spath et al. (16) in a final volume of 200 µL assay buffer containing 0.1% bovine serum albumin. 25I-(Sar, Ile) Ang II was used as the radioligand because it is an antagonist that does not vary in its binding affinity to high- and low-affinity agonist conformations of the angiotensin receptors, and it is resistant to peptidase activity by virtue of the lack of a free amino terminal amino group. To measure the AT1 receptor density, 1 nmol/L 25I-(Sar, Ile) Ang II was used in the presence or absence of the specific AT1 antagonist losartan (1 µmol/L). Nonspecific binding was measured in the presence of 1 µmol/L unlabeled Ang II. The binding assay was performed for 120 min at room temperature and followed by immediate filtration through glass-fiber filters (Whatman GF/C, Hillsboro, OR). The filter-bound radioactivity was counted in a gamma spectrometer (Beckman, LS 3801, Irvine, CA). Receptor affinity and concentration were calcu-
Statistical Analyses

Results were expressed as mean ± SEM. The data was analyzed by one-way analysis of variance followed by the Tukey-Kramer Multiple Comparison test. Differences were considered statistically significant at the \( P < 0.05 \) level.

Results

There were no significant changes in MAP among the four groups (Table 1). The ability of low salt intake or losartan treatment to modulate the renin angiotensin system (RAS) was confirmed by observation of elevated PRA in the three treatment groups compared with that of the control group (\( P < 0.05 \), Table 1).

Although body weight was not significantly different among the four groups, kidney weight and kidney weight/body weight ratio were lower in rats receiving losartan, either alone or in conjunction with a low salt intake, than in control rats (\( P < 0.05 \), Table 2), indicating that blockade of the binding of endogenous Ang II to AT1 receptors retards normal renal growth. A low salt intake alone did not alter the kidney weight or kidney weight/body weight ratio (Table 2), indicating that activation of the endogenous RAS does not necessarily stimulate renal growth.

To determine whether altered renal growth in the losartan-treated rats resulted from changes in cell number or in cell size, renal DNA and protein content and protein-to-DNA ratio were determined (Table 2). DNA and protein content were lower in both losartan-treated groups of rats than in the control rats (\( P < 0.05 \), Table 2), indicating that losartan treatment resulted in decreased renal cell number. Furthermore, the protein/DNA ratio was not altered by losartan compared with that of control rats, indicating that losartan does not affect cell size. In contrast, renal DNA and protein content, and the protein/DNA ratio were not altered by a low salt intake, indicating that sodium deficiency does not change either cell number or cell size (Table 2).

Renal AT1 mRNA content was determined by Northern blot analysis in each of the four groups of rats (Figure 1A). Densitometric analysis (Figure 1B) indicated that the ratio of AT1 mRNA-to-GADPH mRNA was significantly elevated by losartan treatment (0.94 ± 0.07), sodium restriction (1.08 ± 0.05), or the combination of the two (1.16 ± 0.05) compared with the ratio found in controls (0.51 ± 0.06, \( P < 0.05 \)).

Figure 2 shows Scatchard plots of the binding of Ang II to renal tissues from the four experimental groups. The calculated maximal binding (Bmax) and dissociation constants (Kd) of each group are summarized in the Table 3. There is no significant difference in the binding constants among renal preparations from the four groups (Table 3). However, losartan treatment decreased total receptor number (fmol/mg protein) in the presence or absence of salt restriction (\( P < 0.05 \)). Sodium restriction alone tended to increase total receptor number, although the difference from control was not statistically significant.

Figure 3 shows the AT1 receptor density in the kidneys of four groups of rats measured in the presence of 1 nmol/L \(^{125}\text{I}-(\text{Sar}, \text{Ile})\) Ang II. There was a significant decrease in AT1 receptor (\( 1 \mu\text{M losartan displaceable} \)) binding sites in losartan-treated rats (6.2 ± 0.5 fmol/mg protein, \( N = 5 \)), and a significant increase in rats receiving a low sodium diet (29.7 ± 1.6 fmol/mg protein, \( N = 5 \)) compared with controls (18.4 ± 0.4 fmol/mg protein, \( N = 5 \)). The increase in AT1 receptor binding sites induced by sodium restriction was prevented by losartan (15.4 ± 1.6 fmol/mg protein, \( N = 5 \)).

To eliminate the possibility that previously bound losartan inhibits \(^{125}\text{I}-(\text{Sar}, \text{Ile})\) Ang II binding to AT1 receptors, we homogenized and resuspended tissues in 50 mmol sodium acetate (pH 5) to dissociate losartan. We found that, when the tissue was exposed to a mildly acidic wash to eliminate any residual ligand, the specific \(^{125}\text{I}-(\text{Sar}, \text{Ile})\) Ang II binding in losartan-treated rats (8.1 and 10.0 fmol/mg protein, \( N = 2 \)) was still substantially lower than that of rats fed normal sodium diet (23.2 and 20.8 fmol/mg protein, \( N = 2 \)), indicating that it is unlikely that the reduction in the AT1 receptor density results from the previously bound losartan.

Discussion

We have recently demonstrated that low sodium intake upregulates the gene expression of the AT1A receptor in the kidney (4). The study presented here was designed to test the hypothesis that AT1 receptor activation resulting from sodium depletion-induced increases in Ang II leads to an increase in AT1 receptor density that consequently modulates renal growth. We found that the blockade of the binding of Ang II to AT1 receptors by losartan decreases renal mass, confirming the prior report that Ang II mediates normal renal growth through AT1 receptors (8). Surprisingly, however, the renal mass was not altered by sodium restriction, despite the fact that low sodium intake increases both renal AT1 mRNA and receptor density, suggesting that increases in renal mass above a certain normal level requires the activation of multiple factors.

Losartan at a dose of 3 mg/kg/day reduced renal but not somatic growth. Reduced renal growth in the losartan-treated rats does not appear to be caused by tissue hypoperfusion because losartan did not decrease MAP in the present study. Tufro-McReddie et al. have shown that although 27 mg/kg/day of losartan significantly decreased MAP, MAP was still within the range of renal perfusion pressures in which autoregulation of renal blood flow can be achieved (8). Therefore, they also

---

Table 1. MAP and PRA in four groups of rats

<table>
<thead>
<tr>
<th></th>
<th>MAP mm Hg (N = 13 rats/group)</th>
<th>PRA ng/ml/h (N = 6 rats/group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>113 ± 4</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>NS + DUP</td>
<td>115 ± 6</td>
<td>57 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LS</td>
<td>121 ± 3</td>
<td>46 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LS + DUP</td>
<td>113 ± 3</td>
<td>67 ± 4&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> MAP, mean arterial pressure; PRA, plasma renin activity; NS, rats treated with a normal sodium diet; DUP, losartan; LS, rats treated with a low sodium diet. Values are mean ± SE.

<sup>b</sup> \( P < 0.05 \) versus NS.

<sup>c</sup> \( P < 0.05 \) versus LS.
suggested that it is unlikely that hypoperfusion played a major role in determining renal growth impairment observed in losartan-treated weanling rats (8). Their results, along with our observations, indicate that Ang II stimulates renal growth during normal development via the AT1 receptor. We found that both DNA and protein content, but not protein-to-DNA ratio, were lower in losartan-treated rats, indicating that losartan decreases cell proliferation without affecting cell size. These results are in agreement with reports from Tufro-

Table 2. BW, KW, KW/BW ratio, renal DNA and protein content, and protein/DNA ratio in four groups of rats

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>BW (g)</th>
<th>KW (g)</th>
<th>KW/BW (X10^-3)</th>
<th>DNA (mg/kidney)</th>
<th>Protein (mg/kidney)</th>
<th>Protein/DNA (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>13</td>
<td>264 ± 7</td>
<td>1.05 ± 0.02</td>
<td>4.03 ± 0.05</td>
<td>7.2 ± 0.3</td>
<td>120 ± 5</td>
<td>16.7 ± 0.4</td>
</tr>
<tr>
<td>NS + DUP</td>
<td>13</td>
<td>259 ± 5</td>
<td>0.98 ± 0.02^b</td>
<td>3.78 ± 0.04^b</td>
<td>6.0 ± 0.3^b</td>
<td>99 ± 3^b</td>
<td>16.6 ± 0.7</td>
</tr>
<tr>
<td>LS</td>
<td>13</td>
<td>265 ± 5</td>
<td>1.08 ± 0.02</td>
<td>4.07 ± 0.05</td>
<td>7.4 ± 0.4</td>
<td>124 ± 5</td>
<td>17.0 ± 0.8</td>
</tr>
<tr>
<td>LS + DUP</td>
<td>13</td>
<td>262 ± 6</td>
<td>0.99 ± 0.03^b,c</td>
<td>3.80 ± 0.03^b,c</td>
<td>5.8 ± 0.3^b,c</td>
<td>94 ± 4^b,c</td>
<td>16.7 ± 1.1</td>
</tr>
</tbody>
</table>

* BW, body weight; KW, kidney weight; KW/BW, kidney weight to body weight; NS, rats treated with a normal sodium diet; DUP, losartan; LS, rats treated with a low sodium diet. Values are means ± SE. N = number of rats.
^b P < 0.05 versus NS.
^c P < 0.05 versus LS.

Figure 1. Northern blot using 32P-dCTP labeled AT1 cDNA (A, upper panel) to hybridize RNA isolated from kidneys of rats treated with normal sodium diet (NS, lane 1, 2, 3, 4, 5), NS + losartan (DUP, lane 6, 7, 8, 9, 10), low sodium diet (LS, lane 11, 12, 13, 14, 15), LS + DUP (lane 16, 17, 18, 19, 20). Each lane represents one rat (30 pg of total renal RNA). The blots were stripped and rehybridized with 32P-dCTP-labeled glyceraldehyde 3 phosphate dehydrogenase (GAPDH) cDNA probes (A, lower panel). B is densitometric analysis of Northern blot in A corrected for GAPDH in each blot. Results represent mean ± SE. ^* P < 0.05 versus NS.

Figure 2. Scatchard plot of binding of Ang II to renal tissues of rats treated with normal sodium diet (NS, N = 4), NS + losartan (DUP, N = 4), low sodium diet (LS, N = 4), LS + DUP (N = 3). The results of maximal binding (Bmax) and dissociation constants (Kd) from four groups of rats are summarized in the Table 3. Each symbol represents the mean of the Ang II binding of three to four animals in each groups at a particular Ang II concentration.
Table 3. Changes in Ang II receptor density in response to losartan and/or sodium restriction

<table>
<thead>
<tr>
<th></th>
<th>Bmax (fmol/mg protein)</th>
<th>Kd (×10⁻⁷)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>45 ± 2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>NS + DUP</td>
<td>22 ± 4b</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>LS</td>
<td>59 ± 5c</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>LS + DUP</td>
<td>32 ± 6d</td>
<td>1.7 ± 0.3</td>
</tr>
</tbody>
</table>

*Ang II, angiotensin II; Bmax, calculated maximal binding; Kd, dissociation constants; NS, rats treated with a normal sodium diet; DUP, losartan; LS, rats treated with a low sodium diet. Values are means ± SE. N = number of rats.

b P < 0.05 versus NS.
c P = 0.05 versus NS + DUP.
d P < 0.05 versus LS.

Figure 3. Bar graph showing AT1 receptor density in kidneys of four groups of rats measured in the presence of 1 nmol/L Ang II binding site. There are five rats in each group. NS, rats treated with normal sodium diet; DUP, losartan; LS, rats treated with low sodium diet. * P < 0.05 versus NS. + P < 0.05 versus NS + DUP. # P < 0.05 versus LS.

mRNA levels in Sprague-Dawley rats did not change after losartan treatment (27 mg/kg/day) for 3 wk (8). The inconsistency could be the result of differences in the strain of the rat studied, or the duration and dose of losartan given. At present, we have no information to distinguish among these possibilities.

Although currently, the mechanism by which losartan decreases the AT1 receptor density is unclear, several possibilities need to be considered. One is that blockade of the renal AT1 receptor by losartan may interfere with intermediary steps, e.g., posttranscriptional events, needed to transcribe the AT1 mRNA into functional receptor protein. Regulation of posttranscriptional events by agonists has been demonstrated by our laboratory, as well as by others (22,23), and may contribute to regulation of the AT1 receptor and its gene expression in the kidney. Another possible mechanism whereby losartan could decrease AT1 receptor binding is by AT1 receptor occupancy. This does not appear to be the case for two reasons: (1) mild acidic wash to remove previously bound ligand did not alter the losartan induced decrease in Ang II binding, and (2) there was no significant difference in Ang II binding affinity in losartan treated versus control kidneys, which would occur if a competing ligand was bound to the AT1 receptors.

Consistent with our previous finding (4), low sodium intake upregulates AT1 receptor mRNA expression. Similarly, sodium depletion upregulates the AT1 receptor mRNA expression in the adrenal gland (14,24). The mechanism for this upregulation is unclear. Increased PRA by sodium depletion indicates that the circulating RAS is activated. Therefore, it appears that elevated Ang II levels may also modulate AT1 gene expression by a positive feedback mechanism. However, AT1 mRNA levels in rats fed a low sodium diet are also elevated when losartan blocks the binding of Ang II to AT1 receptor, indicating that either losartan has a direct effect on the regulation of AT1 mRNA expression or that other mechanisms exist, e.g., aldosterone effects or other consequences of sodium depletion. Previous studies on the genomic structure and the promoter region sequence of AT1 have shown that several glucocorticoid-responsive elements exist in the 5'-regulatory region and account for stimulation by aldosterone (25,26). It is possible, therefore, that the release of aldosterone induced by low sodium intake accounts for the increase in AT1 mRNA expression in the kidney. This mechanism remains to be explored.

The changes in AT1 receptor density induced by sodium depletion parallel the changes in AT1 mRNA levels, suggesting that increased AT1 mRNA levels lead to an increase in its translational products—AT1 receptors. In support of this, it has been shown that proximal tubule Ang II receptor density is increased with sodium depletion and decreased with sodium loading (27). When an animal is given a low sodium diet, increased circulating or locally produced Ang II binds to an increased number of receptors to modulate proximal tubule functions resulting in sodium retention. It is well established that Ang II is an extremely potent stimulator of Na⁺-H⁺ exchange and bicarbonate reabsorption in the early S1 segments of proximal tubule (28–30). Micropuncture studies have demonstrated that these segments of proximal tubule also contained the highest concentration of Ang II binding site (28,29).

Interestingly, although AT1 receptor density in the kidney was increased by sodium restriction, kidney weight, kidney weight/body weight ratio, and DNA and protein content were not altered. This indicates that activation of the circulating and/or local RASs does not stimulate renal growth. This intriguing finding has led us to propose that the RAS plays distinct roles in normal renal development and altered physiologic conditions. For example, one can speculate that an increase in renal mass above a normal level needs synergistic actions of Ang II and other factors, whereas normal renal development is Ang II-dependent.

In conclusion, we have demonstrated that blockade of endogenous Ang II by losartan results in retardation of the normal renal growth. Although sodium restriction increases both renal AT1 mRNA and receptor protein, renal mass was not altered by sodium depletion. These results suggest that Ang II modu-
lates renal growth during normal development via the AT1 receptor. However, increases in renal mass above normal levels require the activation of multiple factors. An understanding of the role of Ang II in growth regulation of the kidney may provide new information regarding the regulation of blood pressure and the pathogenesis of hypertension and kidney diseases.

Acknowledgments
This study was supported in part by National Institutes of Health Grant HL-52279 and a grant from Merck Research Laboratories to Dr. Donna H. Wang. Dr. Donald J. DiPette is a recipient of a Established Investigator Award from the American Heart Association. We thank Dr. Richard D. Bukoski for his critical review of this manuscript and Wilma Frye for her expert secretarial skills.

References