Renal Angiotensin Receptor Mapping in Obese Spontaneously Hypertensive Rats

Paul Ernsberger, Richard J. Koletsky, Laura A. Collins, and Janice G. Douglas

Obese spontaneously hypertensive rats (SHR) develop nephropathy with severe proteinuria, but lean littersmates do not develop renal disease. Intrarenal angiotensin has been suggested to contribute to nephropathy in other experimental models. We examined the regulation of angiotensin receptors as a reflection of target tissue response to possible changes in the renin-angiotensin system. We visualized angiotensin receptors in kidneys of 6–8-month-old obese SHR and their lean littersmates. Both obese and lean rats were hypertensive as determined by tail-cuff or by direct measurement. Histologic studies showed early glomerular sclerosis in obese but not lean rats. Autoradiographic visualization of angiotensin receptor binding sites in both obese and lean SHR showed glomeruli and medullary rays having the highest levels of binding with additional diffuse labeling in cortex and outer medulla. In obese rats, binding was reduced relative to lean littersmates, particularly in the medulla, while intense binding in glomeruli was preserved. Loss of receptors did not reflect tissue damage, since the medulla showed no pathological changes. Biochemical assays of the binding of subtype-selective antagonists to $^{125}$I-angiotensin sites in intact sections showed that both losartan-sensitive and PD 123319-sensitive sites were decreased in nephrotic obese rats. We conclude that specific binding sites for angiotensin are decreased in obese SHR with early glomerular sclerosis, suggesting that angiotensin receptors may be regulated by pathogenic processes in this model of renal disease. (Hypertension 1993;21:1039–1045)

KEY WORDS • rats, inbred SHR • renin-angiotensin system • DuP 753 • receptors, angiotensin

The obese spontaneously hypertensive rat (obese SHR) is a unique strain of rat with genetic obesity, hyperinsulinemia, glucosuria, and nephropathy superimposed on a genetically hypertensive background.1,2 The obese phenotype is thought to be due to a single recessive gene (fa) related to the Zucker Fatty trait (fa).3,4 The obese SHR has a spontaneous and progressive nephrotic syndrome that is a potential model for human diabetic and hypertensive nephropathies. Lean SHR littersmates, although hypertensive, do not develop proteinuria and nephropathy. The mechanisms causing the nephrotic syndrome are unknown. The intrarenal renin-angiotensin system may participate in the pathogenesis of nephritis.5 Angiotensin II (Ang II) receptors were selected for study because they are the ultimate target of the intrarenal renin-angiotensin system.

Three pharmacologically distinct subtypes of the Ang II receptor have been reported. Ang II subtype 2 (AT$_2$) receptors are not coupled to G-proteins, are sensitive to the selective antagonists CGP 41212A and PD 123319, and are present in the brain, uterus, and adrenal medulla but are absent from the rat kidney.6–8 Ang II subtype 1 (AT$_{1A}$ and AT$_{1B}$) receptors are coupled to G-proteins and both are present in the rat kidney.9–13 AT$_{1A}$ receptors bind losartan with high affinity but recognize neither CGP 41212A nor PD 123319; predominate in vascular smooth muscle, liver, adrenal cortex, and renal glomerular mesangial cells; and have recently been cloned.12,13 AT$_{1B}$ receptors have intermediate affinity for losartan, recognize PD 123319 but not CGP 41212A, and have so far only been detected in the kidney.6–11 The AT$_{1A}$ and AT$_{1B}$ subtypes we have defined are distinct from the AT$_{1A}$ and AT$_{1B}$ isoreceptors recently identified by molecular cloning.14

To characterize the downstream targets of the intrarenal renin-angiotensin system in this model of idiopathic nephropathy, we visualized Ang II receptors in obese SHR and in lean SHR controls. We hypothesized that changes in glomerular function might affect angiotensin production, which may in turn modulate the expression of Ang II receptors in intrarenal target tissues. In addition, to determine whether nephropathy specifically affects only AT$_{1A}$ or AT$_{1B}$ receptors, we measured the inhibition of $^{125}$I-Ang II binding by the selective antagonists losartan and PD 123319.

Methods

Animals

Obese SHR and their lean littersmates were housed in a separate limited-access room of the animal facility. They were provided with standard chow (Ralston-Purina) and water ad libitum unless stated otherwise. Both male and female animals were used at the ages of 8–10 months, corresponding to maximum levels of proteinuria.1,15 Kid-
In Vivo Measurements

Systolic blood pressure was measured in conscious restrained rats by tail-cuff electrosphygmomanometry as previously described. Heart rate was determined from the pressure wave recording. When they were killed, the rats were anesthetized with urethane (1 g/kg i.p.) and cannulated for direct measurement of arterial blood pressure. The kidney was removed, blotted to remove blood, weighed, and then was flash-frozen for
later sectioning, histological studies, and assay of Ang II receptor binding.

**Angiotensin II Receptor Autoradiography**

Autoradiographic assays were performed according to a modification of previously described methods and general methods applicable to receptor autoradiography. Kidneys were sectioned at a thickness of 15 μm in a Hacker-Bright cryostat at -18°C and thaw-mounted onto glass slides coated twice with 1% gelatin and 0.1% chrome alum. Slides were kept at 4°C during sectioning, then dried in a vacuum desiccator for 1 hour, and stored at -70°C for up to 8 weeks. Every 12th section was fixed in buffered formalin without drying and stained with hematoxylin and eosin for anatomic reference.

**TABLE 1. In Vivo Parameters in Obese and Lean Spontaneously Hypertensive Rats**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Body Wt (g)</th>
<th>Kidney Wt (g)</th>
<th>Kidney Wt (g/kg body wt)</th>
<th>Cuff BP</th>
<th>Heart rate</th>
<th>Systolic BP</th>
<th>Mean BP</th>
<th>Diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese SHR</td>
<td>566±21*</td>
<td>2.7±0.1*</td>
<td>4.8±0.2*</td>
<td>182±4*</td>
<td>444±13</td>
<td>151±8</td>
<td>136±8*</td>
<td>115±7*</td>
</tr>
<tr>
<td>Lean SHR</td>
<td>233±3</td>
<td>1.6±0.1</td>
<td>7.0±0.2</td>
<td>192±3</td>
<td>437±12</td>
<td>171±10</td>
<td>160±7</td>
<td>146±7</td>
</tr>
</tbody>
</table>

Wt, weight; BP, blood pressure; SHR, spontaneously hypertensive rat. Data are mean values±SEM for six obese and five lean SHR. *Significant difference between obese and lean SHR; p<0.05 by analysis of variance.
For autoradiographic receptor binding assays, kidney sections were warmed to room temperature inside a vacuum desiccator and incubated for 15 minutes at 20°C in 55 mL Tris-HCl (50 mmol/L, pH 7.4) containing 120 mmol/L NaCl, 5 mmol/L Na₂EDTA, 5 mmol/L MgCl₂, 100 μmol/L bacitracin, 50 μmol/L phenylmethylsulfonylfluoride (PMSF), 10 μmol/L phosphoramidon, and 1 μmol/L leupeptin. The sections were then incubated for 60 minutes in 5.5 mL fresh buffer containing 0.15 to 0.3 nmol/L 

\[ ^{125}I \text{-[Sar}'] \text{Ang II (I25I-Ang II).} \]

Non-specific binding was determined in the presence of nonradioactive Ang II (2 μM). Subtypes were estimated in parallel incubations with the AT₁A-selective antagonist losartan (0.1 μmol/L) or the AT₁B-selective antagonist PD 123319 (1.0 μmol/L). After incubation, the sections were washed twice for 2 minutes in 65 mL ice-cold Tris-HCl buffer (50 mmol/L) at pH 7.4, and quickly dried under a stream of dry cold air passed through calcium sulfate desiccant and a cold trap, then dried under vacuum overnight and placed in x-ray cassettes in contact with Hyperfilm (Amersham) at 4°C. For production of autoradiograms, the sections were warmed to room temperature inside a vacuum desiccator and incubated for 15 minutes at 20°C or the AT₁B-selective antagonist PD 123319. The films were developed with Kodak D19 and fixed. The sections were then incubated for 60 minutes in 5.5 mL fresh buffer containing 0.15 to 0.3 nmol/L 

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Nonspecific binding, defined in the presence of a thousandfold excess of Ang II, was sensitive to 0.1 μmol/L losartan and insensitive to 1.0 μmol/L PD 123319 (Figure 3, hatched region). Less than 40% of specific 

\[ ^{125}I \text{-Ang II binding sites were inhibited by PD 123319 but not 0.1 μmol/L losartan.} \]

The present study demonstrates that in obese SHR with confirmed nephropathy and nephrosclerosis, renal 

\[ ^{125}I \text{-Ang II binding sites are downregulated relative to lean SHR.} \]

The primary site of this downregulation in the obese SHR is in the renal medulla. The loss of specific binding sites is probably not due to tissue damage or cell loss, because no pathological changes were found in the renal medulla. Ang II receptors in the vasa recta of the renal medulla may regulate medullary blood flow20,121 and stimulation of medullary Ang II receptors may exacerbate renal damage.20 Glomeruli, despite extensive damage, continued to express high density of binding sites, and the decrease in the intensity of receptor labeling was localized to the outer medulla, particularly the vasa recta. The diffuse labeling in the cortex and outer medulla was also decreased. The glomeruli of the obese SHR were associated with high densities of autoradiographic grains, despite the extensive damage evident histologically. In most sections, fewer glomeruli were evident in the obese SHR kidney than in lean SHR, although loss of glomeruli clearly did not account for all the loss of binding sites.

Biochemical data with intact kidney sections confirmed that the total density of specific 

\[ ^{125}I \text{-Ang II binding sites per section was decreased in obese relative to lean SHR (Figure 3). In fact, specific binding to kidney sections from obese rats was only about 40% of that shown by lean rats. In both the obese and lean SHR, most of the binding sites were sensitive to inhibition by 0.1 μmol/L losartan and insensitive to 1.0 μmol/L PD 123319 (Figure 3, hatched region). Less than 40% of specific 

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The relative inhibition of 

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FIGURE 2. Autoradiograms of $^{125}$I-angiotensin II (Ang II) binding sites in obese and lean spontaneously hypertensive rat (SHR) kidney. Autoradiographic grains are shown as white on a black background. Panel A: Total binding of $^{125}$I-Ang II to whole kidney section from obese SHR with glomerular sclerosis. Panel B: Nonspecific binding of $^{125}$I-Ang II to a section adjacent to that in panel A incubated in the presence of 2 μmol/L unlabeled Ang II. Panel C: Total binding of $^{125}$I-Ang II to a whole kidney section from a lean SHR labeled and exposed in parallel to the obese SHR section in panel A. Panel D: Nonspecific binding in a section adjacent to that shown in panel C.

densities of Ang II binding sites. The high density of receptors in nephrotic glomeruli may be due to the proliferation of mesangial cells, the primary glomerular cell type expressing Ang II receptors. Losartan-sensitive and PD 123319-sensitive subtypes of Ang II receptor were decreased roughly in parallel, showing that receptor changes were not specific to a single subtype.

The mechanism of the downregulation of Ang II receptors in obese SHR is unknown. The downregulation of Ang II receptors in the medulla may be a reflection of events within the glomerulus controlling Ang II production. Because Ang II can upregulate its own receptor within the kidney, we cannot yet be certain whether downregulation in obese SHR was due to excess Ang II or a relative lack of Ang II. Receptors for Ang II have not yet been characterized in an experimental model of nephritis. However, Ang II receptors are downregulated in insulin-deficient diabetic rats, similar to the present data in hyperinsulinemic diabetic rats. In SHR relative to normotensive control Wistar-Kyoto (WKY) rats, Ang II receptors are upregulated in cell membranes from glomeruli. Autoradiographic studies to compare the distribution of Ang II receptors in SHR and WKY kidneys have not been reported.

The autoradiographic distribution of $^{125}$I-Ang II binding sites in the kidney shown in the present study was consistent with previous reports in demonstrating high densities of sites in the glomeruli and the vasa recta. A significant number of binding sites were also evident in a diffuse distribution across the cortex and outer medulla, presumably corresponding to Ang II receptors in the proximal tubule and descending limb, which make up the majority of the renal mass. Epithelial cells in these regions of the kidney have previously been shown to express functional Ang II receptors. The extended exposure time (35 days) used in the present study may account for the detection of renal Ang II receptors present in low density that were not evident in previous autoradiographic studies.

The present study agrees with radioligand binding data that have shown that a small proportion of $^{125}$I-Ang II binding sites in the kidney are inhibited by low concentrations of PD 123319 and its analogues. Conversely, there is a small proportion of sites resistant to inhibition by nanomolar concentrations of losartan. We have characterized PD 123319-sensitive sites with intermediate affinity for losartan in rat glomerular mesangial cells and in rabbit proximal tubule membranes and have tentatively termed them AT$_1$ receptors. The present study was not designed to fully characterize renal Ang II subtypes in obese and lean SHR, and the physiological role of these putative subtypes
remains to be defined. Nonetheless, the data are consistent with an effect of nephropathy on multiple subtypes of Ang II receptor in obese SHR kidney. Interestingly, treatment of SHR with a selective AT1 receptor antagonist protected SHR from proteinuria and glomerular sclerosis caused by reduced renal mass.29

In the present study, saturation analysis was not performed, so it cannot be concluded whether the decreased binding observed in obese SHR was due to decreased receptor number or to decreased affinity. Since an agonist radioligand was used, a decrease in affinity would correspond to a loss of high-affinity coupled receptors. Thus, obese SHR appear likely to have impaired Ang II–mediated signal transduction. Another limitation of this study is that images of whole kidney were generated using autoradiographic film, so that the expression of Ang II receptors at the single-cell level cannot be determined. Cross-linking methods, as recently described by Sechi and colleagues,8 may allow for a finer resolution of Ang II receptor distribution.

We have previously published quantitative image analysis of autoradiograms of slide-mounted sections of rat kidney.19 This level of analysis is limited to comparing inner and outer cortex and medulla. In the case of Ang II receptors in obese SHR, these regional distinctions are of little value. Cortical receptors include contributions not only from glomeruli but also from renal tubules, which are apparent as a diffuse background in the autoradiograms. Optical density readings of the cortex would represent an average of these two elements. Similarly, medullary optical density would obscure gradations between medullary rays and the surrounding tissue.

The obese SHR represents a unique animal model because it develops severe proteinuria spontaneously while eating regular rat chow,1,15 without the use of toxins or surgical manipulations. Blood pressures are actually lower in obese SHR than in their lean littersmates, indicating that genetic obesity per se does not cause hypertension in this model. Furthermore, the development of nephropathy does not appear to exacerbate hypertension. Conversely, however, hypertension in the obese SHR may contribute to renal disease, since antihypertensive treatment retards its progression.20 The Zucker rat is genetically related to the obese SHR,3,4 and has been proposed as a model of renal injury.31 Unlike the obese SHR, the Zucker rat is not consistently hypertensive.32 Zucker rats express proteinuria and nephropathy, but renal pathology is not as severe as that of the obese SHR, possibly due to lower systemic blood pressure.

The renin-angiotensin system has been implicated in experimental nephritis.5 Treatment with angiotensin converting enzyme inhibitors has been shown to retard the progression of renal disease in humans and in experimental animal models, including the Zucker rat.33 The ameliorative effect of angiotensin converting enzyme inhibitors may be independent of systemic blood pressure reduction.33 Ang II itself causes proteinuria and may induce mesangial hypertrophy.11

We conclude that kidney Ang II receptors are downregulated in obese SHR with nephropathy, consistent with a role of intrarenal Ang II in this model of idiopathic kidney disease. Future studies will examine whether the change in Ang II receptors is an adaptive response or a participant in the pathogenesis of nephropathy.

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