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 littermates 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 littermates. 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 littermates, 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 125I-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 littermates, 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 (AT2) 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 (AT1A and AT1B) receptors are coupled to G-proteins and both are present in the rat kidney.9-11 AT1A 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 AT1B 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 AT1A and AT1B subtypes we have defined are distinct from the AT1A and AT1B isoreceptors recently identified by molecular cloning.14

To characterize the downstream targets of the intrarenal renin-angiotensin system in this model of idio-pathic 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 AT1A or AT1B receptors, we measured the inhibition of 125I-Ang II binding by the selective antagonists losartan and PD 123319.

Methods

Animals

Obese SHR6 and their lean littermates5 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-
FIGURE 1. Photomicrographs of glomeruli and medullary structures in obese and lean spontaneously hypertensive rats (SHR). Unfixed fresh-frozen tissue was sectioned, fixed in formalin, and stained with hematoxylin and eosin. Magnification was ×1,600 for glomeruli and ×625 for medullae before reduction for publication. Panel A: Representative glomerulus from obese SHR. There is glomerular hypercellularity owing to mesangial proliferation. The letters "mp" are placed in the center of a cluster of mesangial cells. The crescentic thickening of Bowman's capsule, as indicated by the paired open arrows, represents early glomerular sclerosis. Adhesions or synechiae between Bowman's capsule and the glomerular tuft are indicated by the two half-filled winged arrows.

Panel B: Representative region of outer medulla from obese SHR, corresponding to areas where a high density of angiotensin receptors were observed. Tubule epithelial cells and interstitial cells both appear normal, and no fibrosis was observed. Panel C (facing page): The lean SHR has no glomerular disease. Bowman's capsule is intact with usual mesangial cellularity and no adhesions between capsule and tuft. Panel D (facing page): Outer medullary regions in lean SHR also were grossly normal.

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>
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<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 Na<sub>2</sub>-EDTA, 5 mmol/L MgCl<sub>2</sub>, 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 <sup>125</sup>I-[Sar']Ang II (<sup>125</sup>I-Ang II). Nonspecific binding was determined in the presence of nonradioactive Ang II (2 µM). Subtypes were estimated in parallel incubations with the AT<sub>1A</sub>-selective antagonist losartan (0.1 µmol/L) or the AT<sub>1B</sub>-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 dipped in distilled water to remove buffer salts. In quantitative biochemical assays, the sections were wiped from the slide with Whatman GF/B filter paper and counted in a gamma counter at 82% efficiency (Packard Cobra). For production of autoradiograms, sections were rapidly 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 before assay. Losartan was obtained from DuPont/Cobra.)

Biochemical data with intact kidney sections confirmed that total density of specific <sup>125</sup>I-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 <sup>125</sup>I-Ang II binding sites were inhibited by PD 123319 but not 0.1 µmol/L losartan. The relative inhibition of <sup>125</sup>I-Ang II binding by these two subtype-selective antagonists did not differ between obese and lean SHR, implying that renal Ang II receptor subtypes may be equally affected by nephropathy.

Materials

<sup>125</sup>I-Ang II was prepared by the chloramine-T method, purified by high-performance liquid chromatography, and stored at −70°C in methanol: 0.1 mmol/L acetic acid: acetonitrile (50:30:20) and diluted in water before assay. Losartan was obtained from DuPont/Merck Pharmaceuticals, Wilmington, Del., and PD 123319 was from Parke-Davis, Ann Arbor, Mich. Other compounds were obtained from Sigma Chemical Co., St. Louis, Mo.

Statistical Analysis

All values are given as mean±SEM. One-way analysis of variance was used to compare group means.

Results

Physiological Findings

Obese SHR weighed 143% more than their lean littermates (Table 1). The kidneys of the obese SHR were heavier than those of the lean SHR, but in proportion to body weight renal mass was reduced, consistent with their chronic kidney disease. Blood pressure measurements in the conscious state by the tail-cuff method showed that both obese and lean SHR are hypertensive, but that blood pressure is actually lower in obese rats. Direct measurement of arterial blood pressure under urethane anesthesia showed that hypertension persisted in the obese and lean SHR. Direct arterial pressures were lower in obese SHR than in lean SHR, confirming tail-cuff measurements.

Histologic Findings

The obese SHR showed early glomerular sclerosis (Figure 1). Fresh-frozen renal sections of obese SHR cortex showed proliferation of glomerular mesangial cells, thickening of Bowman’s capsule, and adhesions between Bowman’s capsule and the glomerular tuft (Figure 1A). In contrast, in the renal medulla (Figure 1B) no abnormalities were found in tubular or interstitial structures. The lean SHR littermates of these rats showed no evidence of glomerular disease (Figure 1C). Bowman’s capsule was intact and delicate with usual mesangial cellularity and no adhesions between capsule and tuft. Comparison of mesangial cellularity between glomeruli in obese and lean rats (compare Figures 1A and 1C) emphasizes the hypercellularity in the obese rats. In lean SHR, the renal medulla also showed a lack of pathological changes (Figure 1D).

Autoradiographic Findings

Autoradiograms of <sup>125</sup>I-Ang II binding sites in both obese (Figure 2A) and lean SHR (Figure 2C) showed intense labeling of glomeruli and the vasa recta of the outer medulla, with diffuse labeling of the remaining cortex and outer medulla. Nonspecific binding, defined in the presence of a thousandfold excess of Ang II, was low and relatively uniform in both obese and lean SHR (Figures 2B and 2D, respectively). The density of specific <sup>125</sup>I-Ang II binding sites was decreased in the obese SHR kidney, despite its greater size. Most of 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 obese SHR kidney than in lean SHR, although loss of glomeruli clearly did not account for all the loss of binding sites.

Biocompatible with intact kidney sections confirmed that the total density of specific <sup>125</sup>I-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 <sup>125</sup>I-Ang II binding sites were inhibited by PD 123319 but not 0.1 µmol/L losartan. The relative inhibition of <sup>125</sup>I-Ang II binding by these two subtype-selective antagonists did not differ between obese and lean SHR, implying that renal Ang II receptor subtypes may be equally affected by nephropathy.

Discussion

The present study demonstrates that in obese SHR with confirmed nephropathy and nephrosclerosis, renal <sup>125</sup>I-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 flow<sup>20,21</sup> and stimulation of medullary Ang II receptors may exacerbate renal damage.<sup>20</sup> Glomeruli, despite extensive damage, continued to express high
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 previously 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$_{1B}$ 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.
We have previously published quantitative image analysis of autoradiograms of slide-mounted sections of rat kidney. 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, without the use of toxins or surgical manipulations. Blood pressures are actually lower in obese SHR than in their lean littermates, 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. The Zucker rat is genetically related to the obese SHR, and has been proposed as a model of renal injury. Unlike the obese SHR, the Zucker rat is not consistently hypertensive. 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. 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. The ameliorative effect of angiotensin converting enzyme inhibitors may be independent of systemic blood pressure reduction. Ang II itself causes proteinuria and may induce mesangial hypertrophy.

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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References
