Enhanced Renal Angiotensin II Subtype 1 Receptor Responses in the Spontaneously Hypertensive Rat

Curtis K. Kost Jr. and Edwin K. Jackson

Results from renal transplantation experiments demonstrate that a renal defect is responsible for the development of hypertension in the spontaneously hypertensive rat (SHR). In addition, studies with inhibitors of the renin-angiotensin system have shown that angiotensin II (Ang II) is required for the development and maintenance of hypertension in the SHR. These observations prompted us to propose the hypothesis that hypertension in these rats is due to an enhanced renal responsiveness to Ang II. The purpose of the present study was to determine whether an enhanced renal responsiveness to Ang II exists in adult (12- to 14-week-old) SHR relative to Wistar-Kyoto control rats. To prevent hypertension-induced changes in renal function in SHR, we maintained both strains in the normotensive state from 4 weeks of age with long-term captopril treatment (100 mg/kg per day). Intrarenal Ang II infusions induced a significantly greater decrease in renal blood flow and glomerular filtration rate and a significantly greater increase in renal vascular resistance in SHR compared with Wistar-Kyoto rats. DuP 753 (Ang II subtype 1 [AT₁] receptor antagonist), but not PD 123177 (Ang II subtype 2 receptor antagonist), blocked the renal responses to Ang II in SHR, suggesting that the enhanced renal responsiveness to Ang II was mediated solely by the AT₁ receptor subtype. Unlike renal responses to Ang II, renal responses to periarterial renal nerve stimulation were similar in both strains, suggesting a selective renal hyperresponsiveness to Ang II in the SHR rather than a general hyperresponsiveness toward all vasoconstrictors. From these studies in chronically captopril-treated rats, we conclude that 1) SHR have a genetically determined, enhanced renal responsiveness to Ang II; 2) the enhanced renal responsiveness to Ang II is mediated by the AT₁ receptor; and 3) renal responses to periarterial nerve stimulation are not significantly enhanced, suggesting a selective hyperresponsiveness to Ang II in the kidneys of SHR. (Hypertension 1993;21:420-431)

KEY WORDS • hypertension, genetic • rats, inbred SHR • angiotensin II • kidney • DuP 753 • losartan • receptors, angiotensin

The spontaneously hypertensive rat (SHR) is widely used in cardiovascular research as a model of human essential hypertension. Interestingly, development of hypertension in SHR can be prevented if SHR are treated from an early age with angiotensin converting enzyme inhibitors. Furthermore, arterial blood pressure can be normalized in adult SHR by inhibiting the renin-angiotensin system with agents such as the nonpeptide angiotensin subtype 1 (AT₁) receptor antagonist DuP 753 (losartan), the renin inhibitor CGP 44099A, a number of angiotensin converting enzyme inhibitors, and active immunization against renin.

The efficacy of interrupting the renin-angiotensin system in SHR is difficult to explain, because SHR do not have elevated plasma renin activity or elevated plasma angiotensin II (Ang II) concentrations relative to their normotensive counterpart, the Wistar-Kyoto (WKY) rat. A possible explanation for the efficacy of interrupting the renin-angiotensin system in SHR, despite normal plasma renin activity, may be that SHR have a more active vascular renin-angiotensin system than WKY rats. However, published data have been inconsistent in this regard, providing equivocal results. An alternate hypothesis is that SHR are simply more responsive to any given Ang II concentration (i.e., the normal circulating and/or local levels of Ang II exert a greater tonic effect in SHR). In support of this latter hypothesis, several previous studies suggest that the vasculature of the SHR is more responsive than that of the WKY rat to the vasoconstrictive effects of Ang II. However, it is difficult to ascertain from those studies whether enhanced responsiveness to Ang II contributes to the development of hypertension or is itself caused by the hypertension. An additional confounding factor is that vascular reactivity to pressor agents is difficult to assess in naive SHR and WKY rats in view of disparate baseline conditions (i.e., mean arterial blood pressure and vascular resistance are obviously much higher in SHR).

In a recent study we addressed the problems of hypertension-induced changes and disparate baselines...
by treating WKY rats and SHR with captopril, an angiotensin converting enzyme inhibitor, from 4 weeks of age to the time of the study at 11 weeks of age. This treatment was effective in normalizing blood pressure in the SHR; thus, baseline conditions were similar in both strains, and pressure-induced alterations were prevented from occurring in the SHR vasculature. In that study, we found that although the rapid-pressor response to Ang II was similar in both strains, the slow-pressor response to Ang II (125 ng/min i.p. over 2 weeks via osmotic minipump) was remarkably greater in SHR than WKY rats. Systolic blood pressure averaged greater than 180 mm Hg in the SHR compared with approximately 120 mm Hg in WKY rats at the end of a 2-week infusion of Ang II. On the other hand, the slow-pressor response to norepinephrine was not significantly different between strains, suggesting a selective hyperresponsiveness to Ang II in the SHR.

In a series of experiments that followed, we found that the enhanced slow-pressor response to Ang II in the SHR could not be accounted for by any augmented effect of Ang II on peripheral neurotransmission or aldosterone release in the SHR. However, experiments in the in situ autoperfused kidneys (Fink and Brody model) of captopril-treated rats revealed that SHR were much more responsive than WKY rats to the renal effects of Ang II. The results of those studies lead us to postulate that hypertension in SHR is due to a greater slow-pressor response to Ang II in the SHR, which in turn is due to a greater renal responsiveness to Ang II.

Although our previous results point toward a renal defect in the SHR as a factor in the development of hypertension, we were concerned about relying solely on data from the in situ autoperfused kidney as evidence for an enhanced renal responsiveness to Ang II in SHR. Accordingly, the purpose of the present study was to investigate, under conditions more physiological and less manipulative than the in situ autoperfused kidney, the enhanced renal responsiveness to Ang II in the SHR and to evaluate the contribution of the newly classified angiotensin receptor subtypes AT1 and AT2 (AT1) in mediating the renal effects of Ang II in the SHR. In addition, renal responses to perirarterial nerve stimulation were compared in SHR and WKY rats to determine whether the responsiveness of the renal vasculature to this very important controller of renal vascular resistance (RVR) is also augmented in SHR. As in our previous studies, all experiments were performed in adult SHR and WKY rats that had been pretreated from 4 weeks of age with captopril to prevent hypertension-induced changes in SHR kidneys and to normalize baseline parameters between the two strains.

**Methods**

**Animals**

Four-week-old SHR and WKY rats were obtained from Taconic Farms (Germantown, N.Y.) and were housed at the University of Pittsburgh Animal Facility where temperature, relative humidity, and the light cycle were controlled at 22°C, 55%, and 7 AM to 7 PM, respectively. Animals were treated in accordance with institutional guidelines. The rats were maintained on Wayne Rodent Blox 8604 (sodium, 135 meq/kg; potassium, 254 meq/kg) (Madison, Wis.) and were given water laced with captopril ad libitum. The amount of captopril added to drinking water was adjusted weekly according to body weight and water intake to provide an approximate dose of 100 mg/kg per day. The captopril solution was prepared fresh daily.

**Experimental Protocol 1: Dose-Related Renal Responses to Angiotensin II in Captopril-Treated Spontaneously Hypertensive and Wistar-Kyoto Rats**

At 12–14 weeks of age, each rat was anesthetized with pentobarbital (50 mg/kg i.p.) and placed on a Deltaphase Isothermal pad (Braintree Scientific Inc., Braintree, Mass.). Body temperature was maintained at 37±0.5°C and continuously monitored by a digital rectal microprobe thermometer (Harvard Apparatus, South Natick, Mass.). A section of PE-240 was inserted into the trachea to facilitate breathing. Three PE-50 cannulas were placed in the left jugular vein and one in the left carotid artery. The carotid cannula was connected to a blood pressure analyzer (Micro-Med, Inc., Louisville, Ky.). One of the jugular catheters was used to administer supplemental anesthetic as needed, and another was attached to a saline-filled syringe to supply boluses for fluid replacement during the experiment. The third jugular catheter was used to deliver the radiolabeled tracers, [3H]para-aminomhippuric acid ([3H]PAH) and [14C]inulin. In these experiments, we chose to estimate renal blood flow (RBF) with [3H]PAH clearance, rather than with a noncannulating blood flow probe, to avoid manipulation of the renal artery and kidney as much as possible.

The abdominal cavity was exposed through a midline incision. A catheter (PE-10) was placed in the left ureter to facilitate urine collection. The left renal artery near the junction of the aorta was very carefully exposed in preparation for insertion of a 30-gauge needle as described below. The radiolabeled tracers, [3H]PAH and [14C]inulin, were dissolved in 5% dextrose, and a bolus intravenous dose (0.75 μCi each) was given followed by a constant rate of infusion (each at 0.05 μCi/20 μL per minute) (model BSP infusion pump, Braintree Scientific). Captopril (30 mg/kg i.v.) was administered over 20 minutes. Saline (0.9%, 50 μL/min i.v.) was infused during a 60-minute stabilization period.

The saline infusion was terminated at the end of the stabilization period. A 30-gauge needle (B-D brand, Fisher Scientific, Pittsburgh, Pa.) attached to Silastic tubing was carefully placed into the left renal artery near its orifice with the abdominal aorta, and an intrarenal arterial (i.r.a.) infusion of saline (0.9%) was initiated at a rate of 50 μL/min, marking the beginning of period A in the experimental protocol 1.

The experimental protocol was divided into three 50-minute infusion periods designated A, B, and C. The first 20 minutes of each infusion period were allowed for equilibration. Urine and midpoint blood samples were collected and hematocrit was determined during the last 30 minutes of periods A, B, and C. Midpoint blood sampling was performed by taking blood samples (200 μL) both 5 and 10 minutes into the 30-minute clearance period and using the sample that best coincided with the midpoint of urine production after adjustment for dead space in the renal system and catheter. Intrarenal arterial infusions were initiated at the beginning of each period and delivered vehicle (0.9% saline with 1:100
Responses to Periarterial Nerve Stimulation in

The abdominal cavity was exposed through a midline incision. A transit-time, noncannulating flow probe (model 2500TR, Packard Instrument Co., Inc., Meriden, Conn.) of plasma and urine samples. Renal plasma flow was corrected for hematocrit for calculation of RBF.

Experimental Protocol 2: Angiotensin Receptor Subtypes in the Spontaneously Hypertensive Rat Kidney

The chronically captopril-treated SHR were prepared for protocol 2 as described above for protocol 1. The experimental protocol was divided into two 50-minute periods. Initiation of intrarenal infusion marked the beginning of each period. In period A, the SHR were randomly assigned to receive intrarenal infusion (50 \( \mu \)L/min) of vehicle, an AT1 angiotensin receptor antagonist (DuP 753, 10 \( \mu \)g/min), or an AT2 angiotensin receptor antagonist (PD 123177, 10 \( \mu \)g/min). The intrarenal infusion of vehicle or antagonist was continued into period B with the addition of Ang II (3 ng/min i.r.a.). The first 20 minutes of each infusion period were allowed for equilibration. Urine and midpoint blood samples (200 \( \mu \)L) were collected and hematocrit was determined during the last 30 minutes of periods A and B. Fluid volume loss during blood sampling was immediately replaced with 0.9% saline (400 \( \mu \)L). Mean arterial blood pressure was monitored throughout the experiment. A large blood volume was collected at the end of the experiment for determination of plasma sodium and potassium concentrations. The left kidney was weighed at the end of the experiment, and renal parameters were normalized to kidney weight.

Statistical Analysis

Analyses were performed on a personal computer using the NUMBER CRUNCHER STATISTICAL SYSTEM software package (Kaysville, Utah). Data from protocols 1 and 3 were analyzed by two-factor analysis of variance (ANOVA) (factor A, strain; factor B, dose of Ang II or frequency of nerve stimulation) with repeated measures on factor B. The dose- or frequency-related effects within a strain were analyzed by one-factor ANOVA with multiple comparisons by Duncan's test. Ang II- or frequency-induced percent changes were compared between strains by unpaired t test. Data from protocol 2 were analyzed by paired and unpaired t test and one-factor ANOVA.

Results

Experimental Protocol 1: Dose-Related Renal Responses to Angiotensin II in Captopril-Treated Spontaneously Hypertensive and Wistar-Kyoto Rats

The effects of intrarenal Ang II (1 and 3 ng/min i.r.a.) were compared in chronically captopril-treated SHR and WKY rats. The mean kidney weight was significantly greater in WKY rats than in SHR (p<0.001;
Kost and Jackson
Enhanced Renal Responses to Ang II

SHR WKY (n=7)

ANOVA RESULTS
STRAIN X DOSE p=.1845
WKY p<.009
SHR p<.008

Figure 1. Graphs show absolute values and percent changes in glomerular filtration rate during intrarenal arterial (i.r.a.) infusion of angiotensin II (Ang II) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats treated with captopril from 4 weeks of age. Values indicate mean±SEM. a, WKY rat value significantly different (p<0.05) from WKY rat baseline; b, SHR value significantly different (p<0.05) from SHR baseline; c, SHR value different (significant at p<0.075) from WKY rat value at same level of treatment. Values obtained from one-factor ANOVA are shown for WKY rats and SHR. Strain × dose interaction value is obtained from two-factor ANOVA with repeated measures on dose of Ang II. See text for a full description of statistical analysis.

Baseline GFR did not differ between strains, and Ang II at 1 ng/min i.r.a. had no effect on GFR in either strain (Figure 1). Analysis by one-factor ANOVA demonstrated a significant effect of Ang II on GFR in both WKY rats (p<0.009) and SHR (p<0.008). Although the strain-by-dose interaction did not reach statistical significance (p=0.1645, two-factor repeated-measures ANOVA), Ang II at 3 ng/min i.r.a. caused a significant reduction in GFR in both strains, with a greater percent decrease observed in the SHR (p=0.0251, t test).

Baseline RBF was not significantly different between strains (p=0.2604, t test). Ang II caused a dose-related reduction in RBF in both strains (p<0.0001, one-factor ANOVA), with a greater overall effect in the SHR (Figure 2). The strain-by-dose interaction was significant (p=0.0277, two-factor repeated-measures ANOVA), and the higher infusion rate of Ang II produced a greater percent reduction in RBF in SHR than WKY rats (p=0.0124, t test).

Baseline RVR was similar in both strains (Figure 3). Ang II caused a dose-related increase in RVR in both WKY rats and SHR (p<0.0001, one-factor ANOVA); however, a greater overall effect was observed in SHR. The strain-by-dose interaction was significant (p=0.0114, two-factor repeated-measures ANOVA), and the percent increase in RVR induced by Ang II at 3 ng/min was greater in SHR (p=0.0366, t test).

Urinary electrolyte excretion, urine flow, and mean arterial blood pressure are shown in Table 1. Baseline sodium and potassium excretion rates did not differ significantly between strains (p=0.2527 and p=0.2832, t test).
test, respectively). There was a tendency for Ang II to produce a greater reduction of urinary sodium excretion selectively in SHR (p = 0.0895, one-factor ANOVA) relative to WKY rats (p = 0.8425, one-factor ANOVA), but the strain-by-dose interaction was not statistically significant (p = 0.3955, two-factor repeated-measures ANOVA). Ang II caused a significant dose-related reduction in potassium excretion of similar magnitude in both SHR (p = 0.0005, one-factor ANOVA) and WKY rats (p < 0.0001, one-factor ANOVA). The strain-by-dose interaction with respect to potassium excretion was not statistically significant (p = 0.9615, two-factor repeated-measures ANOVA). Baseline urinary flow rate tended to be higher in WKY rats, but this was not statistically significant (p = 0.0818). Ang II did not have a significant effect on urine flow in either WKY rats (p = 0.8154, one-factor ANOVA) or SHR (p = 0.1158, one-factor ANOVA), and the strain-by-dose interaction was not significant (p = 0.5991, two-factor repeated-measures ANOVA). Baseline mean arterial blood pressure was similar in both strains (p = 0.7705). There was a slight but significant effect of Ang II to raise mean arterial pressure in WKY rats (p = 0.0020, one-factor ANOVA). However, there was no significant interaction between strain and dose with respect to mean arterial pressure (p = 0.6870, two-factor repeated-measures ANOVA), and the Ang II–induced percent changes in mean arterial pressure of WKY rats were similar to, but less variable than, those observed in the SHR.

Experimental Protocol 2: Angiotensin Receptor Subtypes in the Spontaneously Hypertensive Rat Kidney

The effects of Ang II (3 ng/min i.r.a.) were examined in chronically captopril-treated SHR during intrarenal infusion of either vehicle, the AT1 receptor antagonist DuP 753, or the AT2 receptor antagonist PD 123177. There were no significant differences in mean kidney weight among the SHR randomized to the three treatment groups: Saline, DuP 753, and PD 123177. Although the GFR data tended to be quite variable, baseline values were similar among the groups (p = 0.4486, one-factor ANOVA). Ang II caused a near significant reduction in GFR (Figure 4) in the Saline (p = 0.1195, t test) and PD 123177 (p = 0.0866, t test) groups but not in the DuP 753 group (p = 0.2353, t test). The change in GFR, expressed as a percent of baseline, induced by Ang II significantly differed in the DuP 753 group compared with the Saline and PD 123177 groups (p < 0.05, t test).

Baseline RBF measured during control periods did not differ among groups (p = 0.9204, one-factor ANOVA; Figure 5). Ang II caused a significant and near significant reduction in RBF in the Saline and PD 123177 groups (t test results: p = 0.0361 and p = 0.0724, respectively); however, RBF was not significantly altered by Ang II infusion in the DuP 753 group (p = 0.8493, t test). The Ang II–induced percent changes in RBF were similar in the Saline and PD 123177 groups but differed from the DuP 753 group (p < 0.05, t test).

Baseline RVR was similar in all groups (p = 0.9811, one-factor ANOVA; Figure 6). Ang II caused a significant increase in RVR in both the Saline (p = 0.0489, t test) and PD 123177 (p = 0.0185, t test) groups but not in the DuP 753 treatment group (p = 0.4164, t test). The percent increase of RVR induced by Ang II was similar in the PD 123177 and Saline groups but significantly less in the DuP 753 group (p < 0.05, t test).

Electrolyte excretion rates, urinary flow rates, and mean arterial pressure values are shown in Table 2. Baseline sodium and potassium excretion rates were not significantly different among treatment groups. Ang II failed to induce an effect on sodium excretion in all three groups. Urinary potassium excretion was significantly reduced by Ang II in the Saline (p = 0.0062, t test) and PD 123177 (p = 0.0287, t test) treatment groups, but this effect was not observed in the DuP 753 group (p = 0.5042, t test). In addition, the Ang II–induced percent reduction in potassium excretion was similar in the Saline and PD 123177 groups but differed in the DuP 753 group (p < 0.05, t test). Urinary flow rates were variable, and Ang II did not exert any significant effects on that parameter. Mean arterial pressure increased significantly during the Ang II infusion in both the Saline (p = 0.0217, t test) and PD 123177 (p = 0.0258, t test) groups but not in the DuP 753 group (p = 0.1689, t test).
esis of hypertension in SHR. However, it has been demonstrated repeatedly that renin activity and Ang II concentrations are not elevated in the plasma of SHR relative to WKY rats. Taking this into account, we developed the hypothesis that hypertension in the SHR involves an enhanced responsiveness to Ang II.

In a previous study, we tested the hypothesis that adult SHR have enhanced responsiveness to Ang II by comparing both the rapid- and slow-pressor responses to Ang II in WKY rats and SHR that had been maintained normotensive by long-term captopril treatment from 4 weeks of age. We found that the rapid-pressor responses did not differ between SHR and WKY rats; however, the SHR had a greatly enhanced slow-pressor response to long-term Ang II infusion. This appeared to be a selective hyperresponsiveness to the slow-pressor effects of Ang II in SHR, because the slow-pressor response to norepinephrine was not significantly different between strains.

In subsequent experiments, we focused on determining the cause of the enhanced slow-pressor response to Ang II in the SHR. We considered the possibility that the enhanced slow-pressor effects of Ang II in SHR might involve the sympathetic nervous system. Interestingly, in a recent study, Smits et al administered a ganglionic blocker to Sprague-Dawley rats before and 8 days after initiation of a long-term low-dose Ang II infusion; their results suggest significant involvement of a neural component in the slow-pressor response to Ang II in Sprague-Dawley rats. We previously explored the role of the sympathetic nervous system in mediating the slow-pressor response by administering a long-term low-dose Ang II infusion to captopril-treated SHR and WKY rats that had been sympathectomized with 6-hydroxydopamine. Sympathectomy did not reduce the slow-pressor effect of Ang II in either strain, and SHR still exhibited a significantly greater pressor response to Ang II. Thus, our data indicated that the enhanced slow-pressor effect of Ang II in SHR is not dependent on the sympathetic nervous system.

However, our studies in the in situ autoperfused kidneys of chronically captopril-treated rats revealed...
that the SHR was much more responsive than WKY rats to the renal effects of Ang II,\textsuperscript{15} suggesting that the enhanced slow-pressor response to Ang II in SHR may be due to an enhanced renal responsiveness to Ang II. The present study was designed to expand on our previous work by examining the renal responsiveness to Ang II in captopril-treated SHR and WKY rats using a model that is less invasive compared with the in situ autoperfused kidney. Rats were treated chronically with captopril from 4 weeks of age to approximately 13 weeks of age, at which time the renal responses to intrarenal Ang II infusions were measured using clearance of \( ^3 \text{H}\)PAH and \( ^{14} \text{C}\)inulin as indexes of RBF and GFR, respectively. We intentionally chose not to measure RBF with a flow probe, which would have required additional manipulation of the renal artery and kidney to apply. We found greater renal vascular responses to intrarenal Ang II in the SHR than in WKY rats, particularly at the Ang II infusion rate of 3 ng/min. The Ang II infusions caused significantly greater reductions in GFR and RBF and significantly greater increases in RVR in the SHR compared with WKY rats.

With respect to electrolyte excretion, Ang II tended to decrease sodium excretion to a greater extent in SHR than in WKY rat kidneys; however, this effect did not reach statistical significance \( (p=0.0895) \). In a previous study,\textsuperscript{15} we reported that Ang II caused a greater reduction in sodium excretion in SHR than in WKY rat kidneys; however, this effect did not reach statistical significance \( (p=0.0895) \). In a previous study,\textsuperscript{15} we reported that Ang II caused a greater reduction in sodium excretion in SHR than in WKY rat kidneys. One possible explanation for the discrepancy in our results is that the previous study was performed in the in situ autoperfused kidney model over a wider range of Ang II intrarenal infusion rates. Nonetheless, both of these studies demonstrate enhanced renal vascular responses to Ang II in SHR compared with WKY rats. This hyperresponsiveness to intrarenal infusion of Ang II occurred despite the fact that the SHR were

### TABLE 2. Effect of Angiotensin II on Urinary Electrolyte Excretion, Urine Flow, and Blood Pressure in Spontaneously Hypertensive Rats in the Presence or Absence of Antagonist

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline control</th>
<th>Saline Ang II</th>
<th>PD 123177 control</th>
<th>PD 123177 Ang II</th>
<th>DuP 753 control</th>
<th>DuP 753 Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na\textsuperscript{+} excretion rate (\text{\mu}mol/min per gram kidney)</td>
<td>2.69±0.73</td>
<td>2.20±0.53</td>
<td>1.52±0.37</td>
<td>1.76±0.27</td>
<td>2.59±0.94</td>
<td>3.88±1.26</td>
</tr>
<tr>
<td>Paired t test</td>
<td>\text{n=}5</td>
<td>\text{p=}0.171</td>
<td>\text{n=}4</td>
<td>\text{p=}0.542</td>
<td>\text{n=}4</td>
<td>\text{p=}0.174</td>
</tr>
<tr>
<td>Change in Na\textsuperscript{+} excretion rate (%)</td>
<td>...</td>
<td>-10.7±14.1</td>
<td>...</td>
<td>55.2±57.7</td>
<td>...</td>
<td>65.2±18.8*</td>
</tr>
<tr>
<td>K\textsuperscript{+} excretion rate (\text{\mu}mol/min per gram kidney)</td>
<td>1.84±0.18</td>
<td>1.18±0.14</td>
<td>1.91±0.25</td>
<td>1.17±0.15</td>
<td>1.84±0.26</td>
<td>1.71±0.31</td>
</tr>
<tr>
<td>Paired t test</td>
<td>\text{n=}5</td>
<td>\text{p=}0.0062</td>
<td>\text{n=}5</td>
<td>\text{p=}0.0287</td>
<td>\text{n=}4</td>
<td>\text{p=}0.5042</td>
</tr>
<tr>
<td>Change in K\textsuperscript{+} excretion rate (%)</td>
<td>...</td>
<td>-35.8±5.0</td>
<td>...</td>
<td>-36.8±6.2</td>
<td>...</td>
<td>-6.6±8.6*</td>
</tr>
<tr>
<td>Urine flow (\text{\mu}l/min per gram kidney)</td>
<td>11.9±3.1</td>
<td>12.7±2.9</td>
<td>7.93±2.5</td>
<td>8.87±3.2</td>
<td>9.98±3.9</td>
<td>12.3±4.0</td>
</tr>
<tr>
<td>Paired t test</td>
<td>\text{n=}5</td>
<td>\text{p=}0.2542</td>
<td>\text{n=}5</td>
<td>\text{p=}0.4302</td>
<td>\text{n=}5</td>
<td>\text{p=}0.2233</td>
</tr>
<tr>
<td>Change in urine flow (%)</td>
<td>...</td>
<td>10.8±8.8</td>
<td>...</td>
<td>11.0±11.9</td>
<td>...</td>
<td>83.4±69.3</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>103±4</td>
<td>113±4</td>
<td>106±8</td>
<td>118±9</td>
<td>97±7</td>
<td>107±5</td>
</tr>
<tr>
<td>Paired t test</td>
<td>\text{n=}5</td>
<td>\text{p=}0.0217</td>
<td>\text{n=}5</td>
<td>\text{p=}0.0258</td>
<td>\text{n=}5</td>
<td>\text{p=}0.1689</td>
</tr>
<tr>
<td>Change in MABP (%)</td>
<td>...</td>
<td>9.8±2.9</td>
<td>...</td>
<td>10.9±3.2</td>
<td>...</td>
<td>12.5±7.9</td>
</tr>
</tbody>
</table>

Ang II, angiotensin II; MABP, mean arterial blood pressure. Sodium and potassium excretion rates and urinary flow rates are normalized to gram of kidney weight.

*Significantly different from percent change induced by angiotensin II during saline infusion.
Table 1. Effect of Angiotensin II on Urinary Electrolyte Excretion, Urine Flow, and Blood Pressure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Strain</th>
<th>Vehicle (saline)</th>
<th>Ang II (1 ng/min)</th>
<th>Ang II (3 ng/min)</th>
<th>p (one-way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ excretion rate (µmol/min per gram kidney)</td>
<td>WKY (n=6)</td>
<td>2.14±0.20</td>
<td>2.08±0.22</td>
<td>1.95±0.24</td>
<td>0.8425</td>
</tr>
<tr>
<td></td>
<td>SHR (n=5)</td>
<td>1.76±0.23</td>
<td>1.38±0.24*</td>
<td>0.95±0.27†</td>
<td>0.0895</td>
</tr>
<tr>
<td>Change in Na⁺ excretion rate (%)</td>
<td>WKY (n=6)</td>
<td>...</td>
<td>−1.6±7.6</td>
<td>−0.66±19.5</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>SHR (n=5)</td>
<td>...</td>
<td>−17.8±18.9</td>
<td>−39.1±23.2</td>
<td>...</td>
</tr>
<tr>
<td>K⁺ excretion rate (µmol/min per gram kidney)</td>
<td>WKY (n=6)</td>
<td>1.59±0.13</td>
<td>1.22±0.08§</td>
<td>0.92±0.08§</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>SHR (n=5)</td>
<td>1.37±0.15</td>
<td>1.00±0.20†</td>
<td>0.67±0.14†</td>
<td>0.0005</td>
</tr>
<tr>
<td>Change in K⁺ excretion rate (%)</td>
<td>WKY (n=6)</td>
<td>...</td>
<td>−22.7±3.1</td>
<td>−41.1±4.2</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>SHR (n=5)</td>
<td>...</td>
<td>−28.5±9.2</td>
<td>−52.6±6.9</td>
<td>...</td>
</tr>
<tr>
<td>Urinary flow rate (µL/min per gram kidney)</td>
<td>WKY (n=7)</td>
<td>9.35±1.26</td>
<td>9.69±0.96</td>
<td>9.02±1.25</td>
<td>0.8154</td>
</tr>
<tr>
<td></td>
<td>SHR (n=7)</td>
<td>6.63±0.68</td>
<td>6.67±0.47‡</td>
<td>4.98±0.68‡</td>
<td>0.1158</td>
</tr>
<tr>
<td>Change in urinary flow rate (%)</td>
<td>WKY (n=7)</td>
<td>...</td>
<td>10.1±14.6</td>
<td>2.4±15.3</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>SHR (n=7)</td>
<td>...</td>
<td>8.8±16.5</td>
<td>−19.3±14.1</td>
<td>...</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>WKY (n=7)</td>
<td>105±4</td>
<td>109±4</td>
<td>110±4</td>
<td>0.0020</td>
</tr>
<tr>
<td></td>
<td>SHR (n=7)</td>
<td>107±7</td>
<td>110±5</td>
<td>110±4</td>
<td>0.4947</td>
</tr>
<tr>
<td>Change in MABP (%)</td>
<td>WKY (n=7)</td>
<td>...</td>
<td>4.6±1.3</td>
<td>5.5±1.5</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>SHR (n=7)</td>
<td>...</td>
<td>4.0±3.5</td>
<td>4.0±3.6</td>
<td>...</td>
</tr>
</tbody>
</table>

Ang II, angiotensin II; WKY, Wistar-Kyoto rat; SHR, spontaneously hypertensive rat; MABP, mean arterial blood pressure. Sodium and potassium excretion rates and urinary flow rates are normalized to gram of kidney weight.

• p<0.075, compared with WKY at same treatment level.
• tp<0.05, compared with SHR baseline.
‡ p<0.05, compared with WKY at same treatment level.
§/ †p<0.05, compared with WKY baseline.

Experimental Protocol 3: Frequency-Related Renal Responses to Periarterial Nerve Stimulation in Captopril-Treated Spontaneously Hypertensive and Wistar-Kyoto Rats

RBF and RVR were monitored in chronically captopril-treated SHR and WKY rats during periarterial nerve stimulation at 1 and 3 Hz. The mean kidney weight was significantly greater in WKY rats than in SHR (p<0.0001, 1.52±0.06 and 1.11±0.03 g, respectively). Accordingly, renal hemodynamic parameters were normalized to kidney weight.

Baseline RBF was not significantly different between strains (Table 3). Periarterial nerve stimulation resulted in a frequency-related decrease in RBF of both WKY rats (p<0.0001, one-factor ANOVA) and SHR (p<0.0001, one-factor ANOVA). The strain-by-frequency interaction was not significant (p=0.4562, two-factor repeated-measures ANOVA), and the periaxillary nerve stimulation-induced decreases in RBF were similar between both 1- and 3-Hz frequencies.

There were no strain differences with respect to baseline RVR, and periarterial nerve stimulation caused a significant increase in RVR of both WKY rats (p<0.0001, one-factor ANOVA) and SHR (p<0.0001, one-factor ANOVA; Table 3). The strain-by-frequency interaction was not significant (p=0.4562, two-factor repeated-measures ANOVA), and the periarterial nerve stimulation-induced increases in RVR at both 1 and 3 Hz were similar between SHR and WKY rats.

Discussion

SHR develop hypertension as they age, succumb to a damaged cardiovascular system, and consequently have a much higher rate of premature mortality compared with their normotensive counterpart, the WKY rat. The development of hypertension can be prevented and the survival rate normalized in SHR by long-term treatment with an angiotensin converting enzyme inhibitor from a young age.1-4,6 In addition, blood pressure can be normalized consistently in adult SHR by nearly all treatments that inhibit the renin-angiotensin system.4-8 Thus, it has become increasingly evident that the renin-angiotensin system plays a critical role in the pathogen-
The two concepts discussed thus far, that there is enhanced renal responsiveness to Ang II in SHR and WKY rats. Despite similar baseline plasma renin activity between strains, bolus injections of the AT1 receptor antagonist DuP 753 resulted in a significant increase (approximately 30%) of RBF in the SHR while having no effect on RBF in WKY rats. This suggests that endogenous Ang II contributes to the elevated RVR in adult naive SHR and that it does so by acting at the AT1 receptor.

In addition, a recent abstract by Wood et al lends further support to the hypothesis that a renal hyperresponsiveness to Ang II, mediated by the AT1 receptor, contributes to hypertension in SHR. Low doses of an AT1 receptor antagonist significantly reduced the blood pressure of conscious, freely moving SHR in a dose-related manner if administered by intrarenal infusion; however, these low doses had no significant effect on blood pressure if administered intravenously.

As a final component of the present study, we assessed the selectivity of enhanced renal responsiveness to Ang II versus other pressor agents in SHR. We examined RBF and RVR in chronically captopril-treated WKY rats and SHR during periarterial renal nerve stimulation. The vascular responses we measured, therefore, were due to neuronally released neurotransmitters. Periarterial nerve stimulation at 1 and 3 Hz induced frequency-related reductions in RBF and increases in RVR in both SHR and WKY rats. However, in contrast to the preferential effects of intrarenal Ang II on SHR, the renal responses to periarterial nerve stimulation were of similar magnitude in SHR and WKY rats. This suggests that the enhanced renal responsiveness to Ang II in SHR may be selective to that peptide rather than a general enhanced responsiveness to vasoconstrictors in the SHR kidney.

As mentioned previously, the mechanism by which Ang II exerts a greater renal response in SHR is not known. An obvious possible explanation is that angiotensin receptor density may be greater in the SHR kidney than in the normotensive rat kidney. However, it appears that binding of Ang II to glomeruli from kidneys of 5- and 10-week-old SHR and age-matched Wistar controls is very similar, whereas binding at 15 and 20 weeks is significantly greater in SHR. No significant relation could be found between the age-related change in glomerular Ang II binding and systolic blood pressure in either strain, and actual binding affinities for Ang II in the SHR glomeruli were either similar to or lower than Wistar control values across the age groups of 5, 10, 15, and 20 weeks. In another study, intrarenal arterial administration of Ang II was shown to reduce RBF by 35% more in adult SHR than WKY rats despite similar angiotensin receptor binding characteristics in the kidneys of both strains. The authors of the study were able to abolish the differential effect of Ang II on RBF by treatment with a cyclooxygenase inhibitor and have suggested a defect in the actions of vasodilator prostaglandins in the SHR kidney. At present, we have not tested the effects of a cyclooxygenase inhibitor in our model. Other investigators have demonstrated that Ang II-induced increases in cytosolic calcium and inositol phosphate accumulation were shown to be significantly enhanced in mesangial cells from SHR kidneys compared with WKY.
kidneys, again despite no difference in angiotensin receptor number or affinity. Thus, it appears that a postreceptor signaling defect may be the cause of enhanced responsiveness to Ang II in SHR kidneys.

Our findings are in agreement with the results of the studies mentioned. However, interpretation of results obtained from naive SHR and WKY rats is confounded by problems inherent in comparing responses in SHR and WKY rats, which include 1) baseline differences in pressure and resistance between the two strains and 2) damage of SHR kidneys secondary to a greater renal perfusion pressure. Thus, it is difficult to determine whether data is more relevant if expressed as absolute or percent changes and whether any enhanced renal sensitivity observed might be secondary to renal damage caused by elevated pressure in SHR. To circumvent these problems, the SHR and WKY rats used in our experiments were maintained normotensive by long-term treatment with the angiotensin converting enzyme inhibitor captopril. However, when using this approach, one must accept the possibility that treatment with an angiotensin converting enzyme inhibitor may alter angiotensin receptor density. Fourteen-day treatment with the converting enzyme inhibitor enalapril has been shown to decrease Ang II receptors in the subfornical organ of the SHR brain while producing no significant changes in the subfornical organ of WKY rats. In addition, Ang II receptor binding in aortic smooth muscle cells obtained from long-term captopril-treated SHR was significantly greater than in cells from untreated SHR. Thus, it is possible that treatment with antagonists of the renin-angiotensin system may induce qualitatively or quantitatively different changes in Ang II receptors of SHR and WKY kidneys. Unfortunately, long-term treatment of SHR with most other antihypertensive agents has been ineffective in preventing the development of hypertension and detrimental vascular changes, or those antihypertensive agents might also be expected to alter the level of activation of the renin-angiotensin system. However, similar results obtained in both naive and captopril-treated rats provide strong evidence that SHR have enhanced renal responsiveness to Ang II.

The pathophysiological relevance of the enhanced renal responsiveness to Ang II in SHR is underscored by the results of a previously published study in which a low-dose infusion of Ang II (1 ng/min for 1 week via osmotic minipumps) was administered directly into the left renal artery of captopril-treated SHR and WKY rats. The intrarenal Ang II infusion did not significantly alter systolic blood pressure in the WKY rats; however, systolic blood pressure was significantly increased by 30 mm Hg in SHR. This suggests that an enhanced renal responsiveness to Ang II leads to an enhanced slowpressor response that may contribute to the development of hypertension in SHR.

Compelling evidence for a renal defect as the cause of hypertension in SHR is derived from a number of well-controlled transplantation studies (for review, see References 35 and 36). Several groups have demonstrated that transplantation of renal grafts from SHR donors into normotensive recipients caused hypertension to develop in recipients. Renal grafts from WKY rat donors did not cause hypertension in normotensive recipients. Furthermore, transplantation of WKY rat renal grafts into SHR recipients has been shown to reduce blood pressure in SHR. One criticism of the transplantation studies is that SHR renal grafts may have been damaged secondary to the increased renal perfusion pressure of these animals before transplantation. This objection was addressed by studies in which SHR kidneys were protected from elevated pressure by long-term antihypertensive treatment before transplantation, or renal grafts were obtained from young (4-week-old) SHR that were presumably prehypertensive. In both cases, normotensive recipients of SHR renal grafts developed hypertension, and recipients of WKY rat renal grafts did not.

It has been suggested that defective central nervous system regulation of arterial pressure may be responsible for hypertension in SHR. The results of the renal transplantation studies firmly establish a role for the kidney in the development of hypertension; however, the studies argue against the theory that hypertension is a result of defective central nervous system regulation of arterial blood pressure in SHR because hypertension tracked the SHR kidney, not the central nervous system. The hypothesis that defective central nervous system regulation of renal function is responsible for hypertension in SHR has also been forwarded. However, renal denervation in the adult SHR has little effect on established hypertension, and renal denervation of young SHR will slow, but not cease, the development of hypertension in these rats. Also, a recent renal transplantation and crossbreeding study suggests that genetically determined alterations of renal e-adrenergic receptor numbers do not play a role in development of hypertension in SHR. Finally, the effects of e-adrenergic receptor antagonists on hypertension in SHR have not been consistent; some recent studies have demonstrated that long-term e-adrenergic receptor blockade does not prevent the development of hypertension in SHR whereas earlier studies suggest otherwise. Taken together, these studies in the SHR suggest that a renal defect leads to the development of hypertension and that the renal defect may involve enhanced responsiveness to Ang II but probably does not involve defective central nervous system regulation of renal function. Recent clinical studies also support the idea that enhanced renal responsiveness to Ang II may contribute to the development of hypertension in human subjects as well. In one such study, the systemic and renal hemodynamic effects of low-dose Ang II infusions were compared in normotensive young men with either a positive or negative family history of hypertension. Presumably, the normotensive young men with a positive family history of hypertension are predisposed to developing genetic hypertension and, therefore, represent a prehypertensive class of patients. Subjects with a negative family history were subdivided into two groups: those with a normal body mass (leaner) or those with a body mass similar to subjects with a positive family history of hypertension. Mean arterial blood pressure was similar in the normotensive subjects with a positive family history and in body mass–matched subjects with a negative family history of hypertension; however, pressure was significantly lower in the subjects with normal body mass and a negative family history of hypertension. Nevertheless, baseline parameters such as plasma renin activity, plasma Ang II concentration,
renal plasma flow, and RVR were not significantly different among these groups. Intravenous infusion of Ang II at doses of 0.1 and 0.5 ng/min per kilogram significantly reduced renal plasma flow and increased RVR in subjects with a positive family history of hypertension, whereas these parameters were not significantly altered in the control subjects.

Another recent clinical study indirectly demonstrated an abnormal renal vascular response to Ang II in diabetes with a familial predisposition to hypertension. Acute angiotensin converting enzyme inhibition by captopril administration induced a significant reduction in mean arterial pressure and RVR in diabetics both with and without a familial history of hypertension; however, the reduction in RVR was significantly greater in diabetics with a positive family history of hypertension. Acute administration of the calcium channel blocker nicardipine resulted in equivalent reductions in RVR in the diabetics both with and without a familial predisposition to hypertension, suggesting a renal defect specific to the renin-angiotensin system.

In summary, we have previously demonstrated that chronically captopril-treated SHR and WKY rats respond similarly to acute, rapid-pressor doses of both Ang II and norepinephrine. The long-term slow-pressor effect of norepinephrine is also similar in both strains. However, the long-term slow-pressor response to Ang II is greatly exaggerated in SHR. The mechanism for the exaggerated slow-pressor response in SHR appears to involve the kidney, because long-term low-dose intrarenal infusion of Ang II preferentially causes hypertension to develop in chronically captopril-treated SHR without significantly affecting WKY rats. The concept of a renal defect as the cause of hypertension in SHR is supported by a number of renal transplantation studies. In this study, we have provided evidence that a given concentration of intrarenally administered Ang II will evoke a greater renal vascular response in SHR than WKY rats, suggesting an enhanced renal responsiveness to Ang II in SHR. The renal responses to Ang II in SHR appear to be mediated by the AT1 receptor, because they can be blocked with the AT1 receptor antagonist DuP 753. The enhanced renal responsiveness appears to be selective for Ang II, because we did not observe a greater renal sensitivity to perirterial nerve stimulation in the SHR versus WKY rat kidney. Taken together, our studies indicate that SHR have enhanced renal sensitivity to Ang II and this may contribute to the development of hypertension in these rats.

References

4. Wong PC, Price WA, Chin AT, Duncia JF, Carini DJ, Wester RR, Johnson AL, Timmermans PBMWM: Hypotensive action of DuP 753, an angiotensin II antagonist, in spontaneously hypertensive rats: Nonpeptide angiotensin II receptor antagonists. X. Hyperten-
26. Fontoura BMA, Nussenzveig DR, Timmermans PBMWM, Maack T: DuP 753 is a potent nonpeptide antagonist of angiotensin II.
receptors in isolated perfused rat kidney and cultured renal cells. 


Enhanced renal angiotensin II subtype 1 receptor responses in the spontaneously hypertensive rat.
C K Kost, Jr and E K Jackson

Hypertension. 1993;21:420-431
doi: 10.1161/01.HYP.21.4.420

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/21/4/420

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org//subscriptions/