The spontaneously hypertensive rat (SHR) is an inbred strain that develops high blood pressure with increasing age and is widely used as a model of human essential hypertension. Similar to most patients with essential hypertension, SHR have normal or decreased plasma renin activity (PRA) and are not considered to be a renin-dependent model of hypertension. Nevertheless, angiotensin I converting enzyme (ACE) inhibitors lower blood pressure in SHR and in patients with essential hypertension. ACE inhibitors not only completely prevent the development of hypertension in SHR, if administered from an early age, but also lower blood pressure in adult SHR with established hypertension. In contrast to ACE inhibitors, peptidic angiotensin II (Ang II) antagonists and Ang II antibodies do not consistently lower blood pressure in SHR. In addition, the plasma renin-angiotensin system (RAS) is not elevated in SHR; these observations suggest that the effects of ACE inhibitors could be due to non-Ang II-mediated mechanisms. However, there is also evidence indicating that the effects of ACE blockade are mediated mainly via Ang II. The antihypertensive effects of ACE inhibitors in SHR can be reversed by infusion of Ang II or abolished by a bilateral nephrectomy 24 hours before drug administration. In addition, a recent study has shown that active immunization of SHR against mouse renin lowers blood pressure to normotensive levels. The interpretation of this study is complicated by the development of an autoimmune disease of the kidney. Nevertheless, other recent studies have shown that blood pressure is lowered in SHR after acute administration of novel compounds blocking the RAS at levels other than ACE.
An interesting recent development has been the discovery of a novel class of Ang II antagonists that are completely nonpeptidic and devoid of agonistic properties.\(^8,^9\) Our previous study demonstrated that one of these novel Ang II antagonists (DuP 753) and a rat renin inhibitor (CGP 44099A)\(^11\) lowered blood pressure to a similar extent as the ACE inhibitor enalaprilat after acute administration in SHR.\(^9\) Moreover, pretreatment with the renin inhibitor completely prevented the hypotensive response to the ACE inhibitor and vice versa.

The present study extends these observations and examines the effects of more prolonged treatment with the Ang II antagonist DuP 753 and an ACE inhibitor (benazeprilat). The hemodynamic effects of DuP 753 were studied in bilaterally nephrectomized SHR to determine specificity and the role of the kidney in the antihypertensive response. The compounds were administered in SHR continuously over a 2-day period to determine their maximum effects on blood pressure and heart rate. They were given by intravenous infusion to minimize any influence of their different pharmacokinetic properties. Finally, the biochemical and hemodynamic responses to 7 days of continuous treatment of SHR with the Ang II antagonist or the converting enzyme inhibitor (CEI) were compared. To determine the specificity of the response in SHR, the effects of these different blockers were also studied in normotensive Wistar-Kyoto (WKY) rats. All studies were performed in conscious, freely moving rats to compare the effects of these agents under physiological conditions.

**Methods**

**Animals**

Male SHR and WKY rats were obtained from Iffa Credo, Lyon, France, at an age of 9 weeks (body weight between 230 and 250 g). The rats were allowed 1 week of acclimatization before operation. At the beginning of the experiments, the rats were approximately 11 weeks old (body weight between 250 and 270 g). The animals received a standard rat chow (Nafag, Gossau, Switzerland) and tap water ad libitum. Sprague-Dawley rats were obtained from the CIBA-GEIGY breeding station, Sisseln, Switzerland.

**Blood Pressure and Heart Rate Measurements**

An on-line computerized system was used for continuous intra-arterial measurements of mean arterial pressure (MAP) and heart rate in unrestrained rats. During anesthesia induced by halothane, an arterial catheter was inserted into the aorta via a femoral artery, and a venous catheter was inserted into a femoral vein. Both were exteriorized at the neck. Thereafter, the rats were placed in individual cages and left to recover for 72 hours. To measure MAP and heart rate, the arterial catheter was connected via a swivel to a computer-based system as described previously\(^12\) with some modifications. The signal was preamplified and analyzed via a data logger (DA32 Buxco Electronics, Inc., Sharon, Conn.) and was stored in a personal computer. The quality of the signal was tested via a scrolling monitor and was analyzed on the computer. The top of the swivel was attached to a two-way valve with one arm leading to an infusion pump (B. Braun, Melsungen, FRG). Heparinized saline (2 IU/ml) was infused continuously at a rate of 36 \(\mu\)l/hr into the arterial catheter to prevent occlusion. The other arm of the valve was attached to an Isotec TM pressure transducer (Hugo Sachs Elektronik KG, Freiburg, FRG). This system allowed continuous recording of heart rate and systolic and diastolic pressure and the automatic calculation of MAP. One value of MAP and heart rate was recorded every 2 minutes, and the mean of values recorded was calculated over a 1-hour or a 24-hour period. Values for area under the curve (AUC) were calculated from the values of change in MAP averaged over each hour for the treatment period of interest and were corrected for the values for the pretreatment period (units, mm Hg*hr*). Throughout the experimental procedure the rats were kept in individual cages where they could move freely and had access to food and water. The cages were in a room maintained at 24°C and with a 12-hour light/dark cycle from 7:00 AM to 7:00 PM and 7:00 PM to 7:00 AM.

**Biochemical Methods**

The blood samples for the measurement of plasma concentrations of Ang II were collected as described in detail elsewhere,\(^13\) except that the enzyme inhibitor solution used for the collection of blood also contained a rat renin inhibitor (final concentration, 5 \(\mu\)M CGP 44099A, CIBA-GEIGY, Basel, Switzerland\(^11\)). Blood was collected on ethylenediaminetetraacetic acid to measure plasma renin concentration, plasma renin substrate, and PRA. Blood collected on heparin was used to determine plasma levels of aldosterone, creatinine, urea, electrolytes, osmolality, and hematocrit. Ang II concentrations were measured after extraction from plasma on phenyl-silica gel cartridges and separation of angiotensins by high-performance liquid chromatography as described previously by Nussberger et al.\(^14\) The fractions containing Ang II were measured by radioimmunoassay (RIA) (sensitivity, 1 fmol/fraction). Recovery of the assay is about 90%. Values for unknown samples were corrected for the recovery of internal standards. Determinations of plasma renin concentration were performed by incubation of plasma with an excess of substrate (renin-free plasma from binephrectomized animals) at pH 7.4, 37°C.\(^15\) Plasma renin substrate was determined by incubation of plasma with an excess of renin (isolated from mouse submaxillary glands\(^16\)) at pH 7.4, 37°C.\(^15\) PRA was measured by the angiotensin I (Ang I) antibody trapping method at pH 7.4, 37°C.\(^17\) In the three assays, the Ang I formed was measured by RIA. Aldosterone was determined (without extraction) by RIA with a kit (Coat-A-Count, Diagnostics Products Corp., Los Angeles, Calif.). Plasma electrolytes were measured with a...
Experimental Protocols

Effects of bilateral nephrectomy on response to DuP 753. To evaluate the specificity of the response and the role of the kidney in the antihypertensive effect of the Ang II antagonist, DuP 753 or vehicle was given by an intravenous bolus injection to SHR and WKY rats with intact kidneys or to SHR and WKY rats that had been bilaterally nephrectomized 24 hours before treatment. In the rats with intact kidneys, MAP and heart rate were recorded continuously 24 hours before the compound or vehicle was given. Thereafter, DuP 753 (10 mg/kg, SHR n=7, WKY rats n=5) or vehicle (saline, 0.5 ml/kg, SHR n=4, WKY rats n=4) was given as a bolus intravenous injection, and MAP and heart rate were recorded for a further 24 hours.

In the nephrectomized rats, baseline values for MAP and heart rate were recorded continuously for 24 hours before bilateral nephrectomy. Bilateral nephrectomy was performed under halothane anesthesia. To prevent accumulation of body fluid volume, the nephrectomized rats were deprived of water during the experiments. MAP and heart rate were recorded for another 24 hours after nephrectomy. Thereafter, DuP 753 (10 mg/kg, SHR n=5, WKY rats n=5) or vehicle (saline, 0.5 ml/kg, SHR n=3, WKY rats n=3) was given as a bolus intravenous injection and MAP and heart rate were recorded for a further 24 hours.

To ascertain whether nephrectomy influences the response to a nonspecific vasodilator acting directly on the vasculature, hydralazine was given to both intact (n=6) and nephrectomized (n=6) SHR. The dose (1 mg/kg i.v.) produced a hypotensive response of a similar magnitude to various blockers of the RAS. MAP and heart rate were recorded in both groups for 23 hours before the compound was injected and for 7 hours after the injection.

Dose-response effects of DuP 753 and benazeprilat over 48 hours. To determine suitable doses for a more prolonged study and to compare the maximum hypotensive response induced by agents blocking the RAS at different levels, the blood pressure and heart rate effects of different doses of benazeprilat and DuP 753 were compared after a continuous infusion over a 48-hour period.

Measurement of MAP and heart rate was commenced at least 72 hours after the catheters were implanted. Baseline values for MAP and heart rate were recorded continuously 24 hours before the compounds were given by intravenous infusion (Precisor Infusion Pump, Infors AG, Basel, Switzerland) with an infusion rate of 90 μl/hr for 48 hours. All compounds were dissolved in saline containing 2 IU/ml heparin to prevent occlusion of the infusion catheter. DuP 753 was given in doses of 3 (n=6), 10 (n=9), and 30 (n=7) mg/kg/day, and benazeprilat was given in doses of 3 (n=6) and 10 (n=6) mg/kg/day. The control rats received vehicle only (n=7).

Hemodynamic and biochemical effects of 7-Day treatment with DuP 753 or benazeprilat. To determine whether the hypotensive response to DuP 753 could be maintained during more prolonged treatment, the effects of continuous administration of DuP 753 over 7 days were studied. In addition, the hemodynamic and biochemical consequences of prolonged Ang II antagonism and ACE inhibition were compared in both SHR and WKY rats.

DuP 753 (10 mg/kg/day, SHR n=7, WKY rats n=8), benazeprilat (3 mg/kg/day, SHR n=6, WKY rats n=8), or vehicle (saline, SHR n=6, WKY rats n=8) was given by intravenous infusion (90 μl/hour) over 7 days. MAP and heart rate were recorded continuously for 24 hours before application of the compounds (day -1) and continued for 7 days thereafter.

Body weight of DuP 753–, benazeprilat–, or vehicle-treated rats was recorded at the beginning and during the study. Heart weight was measured at the end of the study, and the ratio of heart weight/body weight (g/kg) was calculated.

Pressor responses to Ang I (1 μg/kg i.v.) and to Ang II (0.3 μg/kg i.v.) were tested in SHR before administration of the compounds on day 0 and at the end of the study on day 7.

At the end of the study (day 7), the rats were disconnected from the system and anesthetized with halothane; blood was collected from the abdominal aorta for the measurement of all biochemical parameters described above. For comparative purposes, blood samples were also collected under the same conditions in the following separate groups of rats for the measurement of PRA and Ang II: binephrectomized SHR (n=7); binephrectomized WKY rats (n=7); renal hypertensive rats (renal hypertension was induced in male WKY rats by a two-kidney, one clip procedure, n=5); sodium-replete Sprague-Dawley rats (n=7); sodium-replete and binephrectomized Sprague-Dawley rats (n=8); sodium-depleted Sprague-Dawley rats (n=8); sodium-depleted and binephrectomized Sprague-Dawley rats (n=6). Blood was collected 36 hours after bilateral nephrectomy. The sodium-depleted rats were maintained on a low-salt diet (9 mmol/kg, Nafag, Gossau, Switzerland) for 14 days.

Statistics

Statistical analysis was performed by computer (Statistical Analyses System Institute Inc., Cary,
Intact Kidneys Bilateral Nephrectomy (NX)

Figure 1. Left panel: Line graphs show effects of a nonpeptidic angiotensin II antagonist (AIIA) (DuP 753, 10 mg/kg) and vehicle (saline, 0.5 ml/kg) in spontaneously hypertensive rats (SHR) (upper graph) and in Wistar-Kyoto (WKY) rats (lower graph) after bolus intravenous injection at 0 hours. AIIA significantly (p<0.05) lowered mean arterial pressure in both SHR (n=7) and WKY rats (n=5). Right panel: Line graphs show effects of an AIIA (DuP 753, 10 mg/kg) and vehicle (saline, 0.5 ml/kg) in binephrectomized SHR (upper graph) (AIIA n=5, vehicle n=3) and in WKY rats (lower graph) (AIIA n=5, vehicle n=3) 24 hours after bilateral nephrectomy. There were no significant differences between the two groups. Values represent mean±SEM of every sixth hour.

N.C.). Analysis of variance (ANOVA) for repeated measurements was used to test for overall differences between groups and time points for the hemodynamic parameters. Model checking was based on the residuals from this analysis. Multiple comparison techniques were used to test for individual differences (f test with Greenhouse-Geisser correction and unpaired t test). Differences in AUC, weights, and biochemical parameters were tested by ANOVA, and individual differences were assessed by multiple comparison techniques (Bonferroni and Dunnett).

All data were expressed as mean±SEM, significance level was taken at p≤0.05.

Results

Effects of Nephrectomy

In rats with intact kidneys, DuP 753 lowered MAP significantly in both SHR and WKY rats (Figure 1, left panel). The response was greater in SHR than WKY rats. MAP was lowered from 154±5 to 127±5 mm Hg (−18%) after 12 hours in SHR and from 115±3 to 105±3 mm Hg (−9%) in WKY rats. MAP of vehicle-treated rats remained stable (MAP before treatment and at 12 hours was 149±4 and 151±3 mm Hg in SHR and 117±3 and 112±5 mm Hg in WKY rats). Values for AUC for the DuP 753-treated rats were significantly different from values for the vehicle-treated rats of both strains (SHR, −512±89 versus 9±12; WKY rats, −226±78 versus 48±72 for DuP 753 versus vehicle, respectively).

PRA fell to very low levels, and Ang II was unmeasurable 36 hours after bilateral nephrectomy in SHR and WKY rats (Table 1). A rapid increase in MAP was observed immediately after bilateral nephrectomy in both rat strains (approximately 30 mm Hg; Figure 1, right panel).

There was no significant change in MAP after a bolus injection of DuP 753 to nephrectomized rats of either strain (Figure 1, right panel). In contrast, hydralazine induced a significant decrease of MAP in both intact and nephrectomized SHR (Figure 2). MAP decreased from 150±5 to 111±3 mm Hg (−26%) after 1 hour in intact SHR and from 180±3 to 134±4 mm Hg (−26%) in nephrectomized SHR.

Dose-Response Effects of DuP 753 and Benazeprilat Over 48 Hours

The maximum antihypertensive effects of DuP 753 and benazeprilat were reached at doses of 10 mg/kg/day and 3 mg/kg/day, respectively (Figure 3). A threefold higher dose of DuP 753 or benazeprilat did not induce significantly greater changes in MAP or AUC. The magnitude of maximum response induced by the two different blockers was similar (approximately 30 mm Hg for the last 24 hours of the treatment), and there were no significant differences.
TABLE 1. Plasma Renin Activity and Plasma Angiotensin II Concentrations in Different Rat Strains Under Various Conditions

<table>
<thead>
<tr>
<th>Rat strains</th>
<th>Plasma renin activity (ng Ang I/ml/hr)</th>
<th>Angiotensin II (fmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=6)</td>
<td>9.24±2.29</td>
<td>6.75±2.93</td>
</tr>
<tr>
<td>AIIA (n=7)</td>
<td>132.4±19.22†</td>
<td>66.95±12.73†</td>
</tr>
<tr>
<td>CEI (n=6)</td>
<td>45.87±13.77</td>
<td>5.75±0.94</td>
</tr>
<tr>
<td>NX (n=7)</td>
<td>0.23±0.01</td>
<td>ND</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n=8)</td>
<td>15.93±4.86</td>
<td>5.88±2.76</td>
</tr>
<tr>
<td>AIIA (n=8)</td>
<td>94.08±18.47*</td>
<td>67.23±19.50†</td>
</tr>
<tr>
<td>CEI (n=8)</td>
<td>77.77±11.60*</td>
<td>8.16±1.76</td>
</tr>
<tr>
<td>NX (n=7)</td>
<td>0.15±0.02</td>
<td>ND</td>
</tr>
<tr>
<td>RHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IN (n=5)</td>
<td>47.34±15.99</td>
<td>65.80±25.03</td>
</tr>
<tr>
<td>NX (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprague-Dawley sodium-replete</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IN (n=7)</td>
<td>31.57±3.35</td>
<td>13.17±3.94</td>
</tr>
<tr>
<td>NX (n=8)</td>
<td>0.03±0.01</td>
<td>ND</td>
</tr>
<tr>
<td>Sprague-Dawley sodium-depleted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IN (n=8)</td>
<td>117.33±13.44</td>
<td>72.04±8.80</td>
</tr>
<tr>
<td>NX (n=6)</td>
<td>0.18±0.02</td>
<td>ND</td>
</tr>
</tbody>
</table>

All values represent mean±SEM. Vehicle, AIIA, or CEI was given for 1 week by continuous intravenous infusion. Ang I, angiotensin I; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; RHR, renal hypertensive WKY rats; vehicle, saline (90 µl/hr); AIIA, angiotensin II antagonist (DuP 753, 10 mg/kg/day); CEI, converting enzyme inhibitor (benazeprilat, 3 mg/kg/day); IN, with intact kidneys; NX, 26 hours after bilateral nephrectomy; ND, not detectable.

*Significant (p<0.05) difference compared with vehicle-treated rats from the same strain.
†Significant (p<0.05) difference compared with CEI-treated rats from the same strain.

FIGURE 2. Line graph shows effects of hydralazine (1 mg/kg) in spontaneously hypertensive rats (SHR) with intact kidneys (IN) (n=6) and in SHR 23 hours after bilateral nephrectomy (NX) (n=6). Compound was given as a bolus intravenous injection in 0.5 ml/kg saline. Mean arterial blood pressure decreased significantly (p<0.05) in both the IN group and the NX group. Values represent mean±SEM of every sixth hour for basal values (first 23 hours) and for points after injection, the first, third, and seventh hour.

FIGURE 3. Line graphs show dose-response effects on mean arterial pressure (MAP) of a nonpeptidic angiotensin II antagonist (AIIA) (DuP 753, upper panel) and a converting enzyme inhibitor (CEI) (benazeprilat, lower panel) in spontaneously hypertensive rats. Vehicle (saline, n=7), AIIA (10 mg/kg/day, n=9; 30 mg/kg/day, n=7), and CEI (3 mg/kg/day, n=6; 10 mg/kg/day, n=6) were given as a continuous intravenous infusion (90 µl/hr) over a 48-hour period. Both doses of AIIA and CEI significantly (p<0.05) lowered MAP for the last 24 hours of treatment period compared with vehicle-treated animals. The threefold higher dose of each compound did not induce significantly greater changes in MAP than the lower dose. Magnitude of hypotensive effect induced by the two compounds was similar. Initial values for MAP were 148±6 and 146±2 mm Hg for AIIA 30 and 10 mg/kg/day; 147±4 and 142±5 mm Hg for CEI 10 and 3 mg/kg/day; 145±4 mm Hg for the vehicle group. Values represent mean±SEM of every fourth hour.

Hemodynamic and Biochemical Effects of 7-Day Treatment

Blood pressure. The hypotensive response to both DuP 753 and benazeprilat was sustained when the treatment was extended for up to 7 days. MAP remained unchanged in the SHR that received vehi-
FIGURE 4. Line graphs show effects on mean arterial pressure (MAP) of continuous administration of vehicle and a nonpeptidic angiotensin II antagonist (AIIA) (DuP 753, upper panel) and of vehicle and a converting enzyme inhibitor (CEI) (benazeprilat, lower panel) in spontaneously hypertensive rats (SHR). Vehicle (saline, n=6), AIIA (10 mg/kg/day, n=7), and CEI (3 mg/kg/day, n=6) were given by a continuous intravenous infusion (90 μl/hour) over 7 days. Values represent mean±SEM of every sixth hour. Statistical analysis is based on means over each day to eliminate daily oscillations. AIIA and CEI significantly (p<.05) lowered MAP compared with baseline values, and effect was maintained over 7 days. In vehicle-treated rats, MAP remained unchanged. There was no significant difference between AIIA- and CEI-treated groups.

FIGURE 5. Line graphs show effects on mean arterial pressure (MAP) of continuous administration of vehicle and a nonpeptidic angiotensin II antagonist (AIIA) (DuP 753, upper panel) and of vehicle and a converting enzyme inhibitor (CEI) (benazeprilat, lower panel) in Wistar-Kyoto (WKY) rats. Vehicle (saline, n=8), AIIA (10 mg/kg/day, n=8), and CEI (3 mg/kg/day, n=8) were given by a continuous intravenous infusion (90 μl/hour) over 7 days. Values represent mean±SEM of every sixth hour. Statistical analysis is based on means over each day. AIIA and CEI significantly (p<.05) lowered MAP compared with baseline values, and effect was maintained over 7 days. In vehicle-treated rats, MAP decreased significantly compared with pretreatment values and also with the values for the vehicle-treated group, and there was no significant difference in the response between the DuP 753- and the benazeprilat-treated groups. Compared with values in the vehicle-treated group, the fall in blood pressure in the WKY rats was less than in SHR. However, the blood pressure of treated SHR was not reduced to as low levels as in treated WKY rats by either compound.

Heart rate. There was no significant effect of either DuP 753 or benazeprilat on heart rate in SHR or WKY rats (Figure 6). In both strains, heart rate was higher during the dark cycles (active state), and the treatments did not influence this diurnal rhythm.

Pressor responses to angiotensin I and angiotensin II in spontaneously hypertensive rats. Before starting the treatments, the pressor responses to Ang I and Ang II were similar in all three groups (Table 2). After 7 days of treatment, the pressor response to Ang I was significantly inhibited in both the DuP 753 (82%) and the benazeprilat (77%) groups and unchanged in the
FIGURE 6. Line graphs show effects on heart rate of continuous intravenous (90 μl/hr) administration of vehicle (saline), a nonpeptidic angiotensin II antagonist (AIIA) (DuP 753), and a converting enzyme inhibitor (CEI) (benazeprilat) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Vehicle (saline, n=6 SHR and n=8 WKY rats), AIIA (10 mg/kg/day, n=7 SHR and n=8 WKY rats), and CEI (3 mg/kg/day, n=6 SHR and n=8 WKY rats) were infused continuously over 7 days. There was no significant effect of either AIIA or CEI on heart rate in SHR or WKY rats. Treatments did not influence diurnal rhythm. Values represent mean±SEM of every sixth hour. Whole numbers represent midday.

Plasma renin concentration increased significantly after treatment with DuP 753 in both SHR (sevenfold, Figure 7) and WKY rats (fourfold, Figure 8). After treatment with benazeprilat, plasma renin concentration tended to increase in SHR (Figure 7) and increased significantly in WKY rats (threefold, Figure 8). Similar increases in PRA were observed (Table 1). The increase in plasma renin concentration tended to be greater in the DuP 753–treated rats than in the benazeprilat-treated rats of both strains (Figures 7 and 8), and the increase in PRA was significantly greater in the DuP 753– than in the benazeprilat-treated SHR (Table 1).

Plasma renin substrate tended to decrease in the DuP 753–treated SHR (Figure 7) and was significantly decreased by both treatments in WKY rats (Figure 8).

Plasma concentrations of Ang II were significantly increased in the DuP 753–treated rats compared with the vehicle- and benazeprilat-treated rats in both rat strains (SHR, 10-fold, Figure 7; WKY rats, 11-fold, Figure 8). The plasma concentrations of Ang II were increased in the DuP 753–treated rats to levels similar to those in renal hypertensive rats and sodium-depleted Sprague-Dawley rats (Table 1). No significant reduction in plasma concentrations of Ang II were observed after treatment with benazeprilat in either rat strain. However, Ang II was reduced to unmeasurable levels 36 hours after bilateral nephrectomy in the various rat models (Table 1).

Plasma concentrations of aldosterone did not change after either treatment in SHR (Figure 7) or WKY rats (Figure 8). Plasma concentrations of aldosterone were significantly lower in SHR (50%) than in WKY rats (Figures 7 and 8).

TABLE 2. Pressor Responses to Angiotensin I and Angiotensin II Before (Day 0) and After (Day 7) Continuous Administration of Vehicle, an Angiotensin II Antagonist, or a Converting Enzyme Inhibitor in Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Angiotensin I (1 μg/kg)</th>
<th>Angiotensin II (0.3 μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
</tr>
<tr>
<td>Vehicle (saline, 90 μl/hr) (n=6)</td>
<td>53.3±4.3</td>
<td>56.0±3.3</td>
</tr>
<tr>
<td>AIIA (10 mg/kg/day) (n=7)</td>
<td>44.4±4.2</td>
<td>7.6±2.9*</td>
</tr>
<tr>
<td>CEI (3 mg/kg/day) (n=6)</td>
<td>50.8±4.2</td>
<td>11.8±5.3*</td>
</tr>
</tbody>
</table>

All values represent mean±SEM of change in mean arterial pressure. AIIA, angiotensin II antagonist (DuP 753); CEI, converting enzyme inhibitor (benazeprilat).

*Significant (p≤0.05) difference compared with day 0.
potassium between SHR and WKY rats. However, plasma concentrations of creatinine were significantly lower and urea significantly higher in SHR than in WKY rats.

Heart and body weights. Both DuP 753 and benazeprilat significantly reduced heart weights in SHR (heart weights were 3.721±0.041, 3.730±0.077, and 3.995±0.031 g/kg body weight for the DuP 753, benazeprilat, and vehicle group).

Heart weights tended to be decreased in WKY rats, but this was only statistically significant in the benazeprilat-treated group (heart weights were 3.306±0.068, 3.240±0.032, and 3.430±0.047 g/kg body weight for the DuP 753, benazeprilat, and vehicle group). Heart weights were significantly higher in SHR than in WKY rats. DuP 753 or benazeprilat had no significant effects on body weights in either SHR or WKY rats.
In the present study, we compared the effects of continuous treatment with an ACE inhibitor, benazeprilat, or an Ang II antagonist (DuP 753), over a 48-hour period on blood pressure and heart rate (continuously monitored) in conscious, freely moving SHR. The results show that the magnitude of the maximum antihypertensive response induced by the two different modes of interference with the RAS was similar. The treatment was extended for up to 7 days to determine whether the antihypertensive effect could be sustained over a longer time period and to compare the hemodynamic and biochemical consequences of prolonged Ang II antagonism and ACE inhibition. The initial responses to DuP 753 and to benazeprilat were virtually identical, and the hypotensive effects of both blockers were sustained over the 7 days of treatment. DuP 753 and benazeprilat were also effective in lowering blood pressure in WKY rats, the normotensive control strain. The antihypertensive effects of both blockers were more pronounced in WKY rats than in SHR, although plasma concentrations of renin and Ang II were similar in the two strains. However, the blood pressure of the treated SHR was not reduced to levels as low as in the treated WKY rats, indicating that there was not a complete normalization of blood pressure in SHR.

A recent study comparing the same Ang II antagonist with another ACE inhibitor (captopril), showed that an acute single administration of the Ang II antagonist induced a hypotensive response of greater magnitude than the ACE inhibitor in sodium-replete SHR but not in sodium-depleted SHR. We did not observe this difference in our present or previous studies, even though the SHR were sodium-replete. This discrepancy may be due to different pharmacokinetic properties and distribution of the compounds used in these studies. The reason that we chose to give the compounds by continuous infusion in our study was to minimize the influence of any pharmacokinetic differences between the compounds.

Our study confirms others indicating the major mechanism whereby ACE inhibitors lower blood pressure in SHR is by the inhibition of the formation of Ang II. Other possible mechanisms such as inhibition of bradykinin degradation would appear to be of minor significance under these experimental conditions. The RAS appears to play an important role in the maintenance of the high blood pressure in SHR. It has been generally assumed that the plasma RAS is not important in SHR since it is not elevated above levels in WKY rats. However, considering the higher blood pressure in SHR, it could be expected that the plasma RAS should be suppressed to lower levels than in WKY rats by the negative feedback of the higher pressure on renin release. Therefore, although the plasma RAS is not elevated in SHR, it may be still too high for this level of blood pressure.

Evidence supporting a role for circulating renin of renal origin or the intrarenal RAS in the maintenance of high blood pressure in SHR comes from observations that the response to ACE inhibition is abolished after bilateral nephrectomy. This was also demonstrated with the Ang II antagonist used in this study. In addition, another recent study with the same Ang II antagonist reports similar findings. These studies exclude a direct vasodilatory effect of the Ang II antagonist on the vasculature and probably also an action via the central nervous system. Although it is unlikely, the possibility cannot be excluded that nephrectomy leads to a suppression of the extrarenal RAS. However, at least after acute treatment, the extrarenal RAS does not appear to be an important target for the acute antihypertensive response. If the production of Ang II within vascular tissue is important, the nephrectomy experiments indicate that Ang II is mainly formed from renin of renal origin. As well as circulating renin, the local RAS within the kidney may be important for the maintenance of high blood pressure in SHR. Indeed, there is considerable evidence that the kidney plays a central role. Hypertension is transferred to WKY rats by transplantation of SHR kidneys and vice versa. In addition, a number of abnormalities in renal function, which normalize as blood pressure increases, are observed in young SHR, indicating that an elevated blood pressure may be necessary to compensate for the decreased renal function in
SHR.20 The exact nature of the renal defect is not clear, but there is evidence to suggest that the RAS could be involved.20,21

Neither benazepril nor DuP 753 had any significant effect on heart rate or on the diurnal rhythm of heart rate. This is consistent with many experimental and clinical studies showing that ACE inhibitors do not induce reflex tachycardia.1 Our study indicates that Ang II antagonists also do not induce reflex tachycardia. Both compounds significantly reduced cardiac hypertrophy to a similar extent in SHR after only 1 week of treatment. ACE inhibitors, as a class of drugs, have been consistently shown to reduce cardiac hypertrophy.22 Our studies suggest that Ang II antagonism may be as effective as ACE inhibition in this respect.

Changes were as predicted in plasma components of the RAS after 1 week of treatment with DuP 753 if the negative feedback of Ang II on renin release was blocked. Both renin and Ang II concentrations were significantly elevated. Plasma concentrations of angiotensinogen tended to decrease, probably due to the higher consumption of substrate by the elevated renin. Benazepril inhibited similar changes in renin and substrate. Plasma concentrations of Ang II were lower in SHR and WKY rats than in the other rat models. Benazepril did not reduce Ang II below levels measured in the vehicle-treated SHR and WKY rats. In contrast, plasma Ang II was reduced to unmeasurable levels after bilateral nephrectomy in all rat models. However, the amount of Ang II in relation to the renin concentration was reduced in the benazepril-treated rats. The observation that the pressor response to Ang II was substantially blocked to the same extent as in the DuP 753–treated rats provided additional evidence that the formation of Ang II from Ang I was indeed inhibited in the benazepril-treated rats. It is interesting that the fall in blood pressure was sustained, despite the apparently normal levels of Ang II in the benazepril-treated rats. We obtained similar results during 14 days of continuous treatment of sodium-depleted marmosets with a renin inhibitor.13 Our studies, in confirmation of others,23 indicate that there is a dissociation between the plasma concentrations of Ang II and the blood pressure decrease after prolonged blockade of the RAS.

Although the Ang II antagonist used in this study has been shown to block Ang II–stimulated release of aldosterone from adrenal capsular cells in vitro24 and in normotensive rats in vivo,25 we did not observe any effects of DuP 753 on plasma concentrations of aldosterone. However, benazepril also had no effect. This indicates that other factors played a more important role in controlling the release of aldosterone than Ang II. The effects of the blockers may have been masked by adrenocorticotropic hormone–induced release of aldosterone during blood collection. Although plasma concentrations of Ang II and renin were similar in our study, plasma values for aldosterone were lower in SHR than in WKY rats, which has been reported previously.26

It is interesting to compare the results of the present study with those from the study on renin immunization in SHR.7 The hemodynamic and the biochemical responses to renin immunization, Ang II antagonism or ACE inhibition are quite comparable, indicating that the major effects of the immunization were due to the inhibition of renin and not to the renal disease. Plasma urea concentrations were significantly increased in the immunized rats, whereas neither DuP 753 nor benazepril had any effects on plasma urea or creatinine concentrations. The increased urea in the immunized rats was probably a consequence of the renal disease. The renin immunization had no effect on sodium excretion and balance. Similarly, no effects on plasma electrolytes or hematocrit were detected in the present study with the ACE inhibitor or the Ang II antagonist, despite a recent report that DuP 753 is a potent proximal tubule diuretic.27

The Ang II antagonist used in the present study (DuP 753) is selective for one receptor subtype (the AT₁ receptor28). Since both the blood pressure and heart rate responses to DuP 753 were similar to those induced by benazepril, it would appear that the major effects of Ang II on blood pressure in SHR are mediated via this receptor subtype. It seems that the negative feedback mechanism of Ang II on renin release is also mediated via the AT₁ receptor. The function of the other subtype (AT₂) is not clear. Since the AT₂ receptor subtype is not blocked by DuP 753 and Ang II concentrations were markedly increased, the functions mediated via this receptor might be enhanced in the DuP 753–treated rats. However, no unexpected effects were observed over a 7-day period.

In summary, when the influence of their different pharmacokinetic properties is minimized, a nonpeptidic Ang II antagonist, selective for the AT₁ receptor subtype, appears to be as effective as an ACE inhibitor in lowering blood pressure in conscious, freely moving SHR. The effects of the Ang II antagonist were sustained for at least 1 week, despite a marked increase in renin release. The Ang II antagonist had no obvious adverse effects. The antihypertensive effects of ACE inhibition in SHR appear to be mainly due to the blockade of the RAS. Ang II appears to play an important role in the maintenance of the high blood pressure in SHR that is mediated via the AT₁ receptor subtype.

Acknowledgments

We thank Paul-André Salamin and Gudrun Feige for the statistical analyses. We gratefully acknowledge the excellent technical assistance of Hans-Peter Müller, Ursula Müller, Liliane Hartmann, Michele Artigot, and Marlis Fischer.

References

Prolonged angiotensin II antagonism in spontaneously hypertensive rats. Hemodynamic and biochemical consequences.

B Bunenburg, C Schnell, H P Baum, F Cumin and J M Wood

Hypertension. 1991;18:278-288
doi: 10.1161/01.HYP.18.3.278

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/18/3/278

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/