Hypertension

Effect of Captopril and Hydralazine on Arterial Pressure–Urinary Output Relationships in Spontaneously Hypertensive Rats

ROBERT L. KLINE AND PAUL F. MERCER

SUMMARY Angiotensin I converting enzyme inhibitors are typically classified as peripheral vaso-dilators. We studied the effect of captopril and a known vasodilator, hydralazine, on arterial pressure–urinary output relationships in adult spontaneously hypertensive rats to determine whether these drugs produced similar changes in this relationship. Tail-cuff pressure and 24-hour urine output and sodium excretion were measured under steady state conditions during ingestion of tap water or saline (1% NaCl) ad libitum. Sodium intake increased seven to nine times when rats drank saline, but in the absence of drug treatment, tail-cuff pressure was not altered significantly (water, 213 ± 3 vs saline, 220 ± 5 mm Hg). Daily administration of captopril (100 mg/kg p.o.) or hydralazine (15 mg/kg p.o.) for 2 weeks lowered tail-cuff pressure significantly (175 ± 3 and 166 ± 3 mm Hg, respectively; \( p < 0.01 \)) while rats drank tap water. Continued administration of hydralazine plus 2 weeks of drinking saline did not alter tail-cuff pressure (162 ± 4 mm Hg), but with the addition of saline during captopril treatment, tail-cuff pressure was elevated significantly (210 ± 5 mm Hg; \( p < 0.01 \)). Thus, hydralazine produced a parallel shift of the arterial pressure–urinary output relationship along the pressure axis. In contrast, captopril produced a marked change in the slope of this relationship, making arterial pressure extremely salt-sensitive. The results suggest that the two drugs have different effects on the mechanisms that contribute to the long-term control of arterial pressure. The changes associated with captopril treatment are consistent with the removal of the normal renal effects of the renin-angiotensin system, rather than with a nonspecific peripheral vasodilation.

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ALTHOUGH numerous types of drugs are effective in treating primary hypertension, the physiological changes leading to the decrease in arterial pressure during administration of these drugs are not well documented. One of the major obstacles to understanding the mechanism of action of antihypertensive agents is the failure to adequately describe the pathophysiology of hypertension. Using a systems analysis approach, Guyton et al. have provided a useful conceptual framework for understanding the long-term control of the circulation in normal and abnormal conditions, including hypertension. According to the predictions of their model, the ability of

the kidneys to excrete sodium and water is a primary determinant of the long-term steady state level of arterial pressure. The importance of pressure-diuresis and pressure-natriuresis to the long-term control of arterial pressure has recently been reviewed. This relationship between arterial pressure and excretion of sodium and water by the kidneys, called the renal function curve (RFC), has also been described for several models of experimental hypertension. Clearly, many factors, including antihypertensive drugs, are capable of changing the position and slope of the RFC and thus are capable of changing the steady state level of arterial pressure. To date, this concept has not been used to characterize the effects of antihypertensive drugs in basic or clinical research (i.e., to determine how commonly used antihypertensive drugs alter the RFC). Such information may help in understanding the mechanism of action of these agents.

We have used this approach in a simple experimental paradigm to characterize the effect of a vasodilator, hydralazine, and a converting enzyme inhibitor, capto-
hil, on the renal function curve of adult spontaneously hypertensive rats (SHR). The results indicate that these two drugs produce different effects on the RFC, suggesting that a direct, generalized reduction of vascular resistance is not the primary mechanism of action of captopril.

Materials and Methods

Fifteen male SHR (Charles River Breeding Laboratories, Montreal, Quebec, Canada) were maintained on a diet of tap water and Purina rat chow (St. Louis, MO, USA) ad libitum in individual metabolism cages until 16 weeks of age, when rat chow pellets were replaced with pulverized rat chow so that food intake could be measured. Rats were handled repeatedly during this time and were fully accustomed to the restraining cage and equipment used to measure tail-cuff pressure (IITC, Woodland Hills, CA, USA). All rats were subsequently cycled through a schedule of treatments (each 2 weeks in duration; Table 1) beginning at 17 weeks of age, in which steady state measurements were obtained during normal salt intake (rat chow plus tap water ad libitum) or high salt intake (rat chow plus 1% NaCl drinking fluid ad libitum). Tap water was not available to the rats during the high salt regimen.

Food intake, fluid intake, and urinary output were measured over a 24-hour period twice each week. Urinary sodium concentration was measured using a flame photometer (Model FLM3; Radiometer, Copenhagen, Denmark) and used to calculate sodium excretion. Tail-cuff pressure was measured twice a week using a protocol described previously. Arterial pressure was not measured on days that were used to monitor intakes and outputs. Hydralazine (15 mg/kg p.o.) and captopril (100 mg/kg p.o.) were given each morning in tap water (0.5 ml/100 g body weight) using a standard pediatric feeding tube (5 F; 45 cm long). These doses have been shown to lower arterial pressure significantly when given chronically to SHR. On days when tail-cuff pressure was measured, the drugs were given after the measurement was made.

Results obtained during the second week of each cycle were averaged to give values for that week. Data were expressed as means ± SE. There was little difference between values for Week 1 and Week 2 in any given condition, indicating rapid adjustment to new steady state conditions. Data within the tap water and 1% NaCl conditions were analyzed using an analysis of variance (ANOVA) with repeated measures (drug treatment) followed by Newman-Keuls tests for differences between individual means. For the arterial pressure data, an ANOVA for repeated measures (drug treatment) under different conditions (tap water vs 1% NaCl) was done, followed by paired t tests of appropriate comparisons. To construct estimated RFCs, data for average urine output and urinary sodium excretion were plotted against average tail-cuff pressure during tap water and 1% NaCl conditions. The slope of the estimated RFC for each animal was calculated assuming a linear relationship between the two data points. This was a reasonable assumption for the range of sodium intake used in this study, based on a previous detailed study of RFCs in the SHR. The reciprocal of the slope was used to express the sensitivity of arterial pressure to dietary sodium. A value was calculated for each animal, averaged, and tested for statistical significance among groups using ANOVA and Newman-Keuls tests. Analyses yielding p values below 0.05 were considered to indicate statistically significant differences among treatments or between conditions.

Results

Complete results for both tap water and 1% NaCl conditions for each treatment were obtained in 10 rats. Table 2 summarizes the input-output data while rats were drinking tap water. After 2 weeks of either no drug treatment, captopril treatment, or hydralazine treatment there were no significant differences in food or water intake among the groups, although urine output was significantly elevated during captopril treatment when compared with control or hydralazine treatment values, and sodium excretion during hydralazine treatment was increased significantly compared with control values. Tail-cuff pressure was decreased significantly during captopril treatment or hydralazine treatment.

### Table 1. Experimental Protocol Used to Cycle SHR Through Normal and High Salt Intake Conditions and Drug Treatments

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Drinking fluid</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>normal salt diet</td>
<td>Tap water</td>
</tr>
<tr>
<td>2</td>
<td>normal salt diet</td>
<td>Tap water</td>
</tr>
<tr>
<td>3</td>
<td>high salt diet</td>
<td>1% NaCl</td>
</tr>
<tr>
<td>4</td>
<td>high salt diet</td>
<td>1% NaCl</td>
</tr>
<tr>
<td>5</td>
<td>high salt diet</td>
<td>1% NaCl</td>
</tr>
<tr>
<td>6</td>
<td>normal salt diet</td>
<td>Tap water</td>
</tr>
</tbody>
</table>

Each cycle lasted 2 weeks; rats had ad libitum access to normal rat chow at all times.

### Table 2. Effect of Chronic (2 wk) Administration of Captopril or Hydralazine in 10 Adult SHR Fed Normal Salt Diet (Drinking Tap Water Ad Libitum)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Captopril (100 mg/kg)</th>
<th>Hydralazine (15 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/24 hr)</td>
<td>23.2 ± 0.7</td>
<td>22.7 ± 0.5</td>
<td>21.0 ± 0.6</td>
</tr>
<tr>
<td>Water intake (ml/24 hr)</td>
<td>44.5 ± 1.5</td>
<td>47.7 ± 1.5</td>
<td>43.3 ± 2.3</td>
</tr>
<tr>
<td>Urine volume (ml/24 hr)</td>
<td>13.7 ± 1.6</td>
<td>18.5 ± 4.1*</td>
<td>15.5 ± 1.6*†</td>
</tr>
<tr>
<td>Sodium excretion (mmol/24 hr)</td>
<td>1.48 ± 0.14</td>
<td>1.59 ± 0.07*</td>
<td>1.76 ± 0.13*†</td>
</tr>
<tr>
<td>Tail-cuff pressure (mm Hg)</td>
<td>213 ± 3</td>
<td>175 ± 3*</td>
<td>166 ± 3*†</td>
</tr>
</tbody>
</table>

Values are means ± SE of average values measured twice during the second week of each cycle (see Table 1). *p < 0.05, compared with control values (by Newman-Keuls test after repeated-measures ANOVA). †p < 0.05, compared with captopril values (by Newman-Keuls test after repeated-measures ANOVA).
When rats were permitted free access to 1% NaCl drinking fluid, sodium intake was increased approximately seven to nine times. During captopril treatment the rats drank significantly less of the 1% NaCl solution than during the control or hydralazine conditions. Food intake was increased slightly while rats were receiving captopril, but urine volume and sodium excretion were significantly less during captopril treatment than during either control or hydralazine conditions (Table 3). Urine volume and sodium excretion were also significantly less during hydralazine treatment when compared with the control values. Tail-cuff pressure during captopril treatment was slightly but significantly lower when compared with the control value, while tail-cuff pressure during hydralazine treatment was significantly lower when compared with either the control or captopril treatment values. By comparing the tap water and 1% NaCl conditions, it can be seen that increasing the intake of NaCl did not significantly alter tail-cuff pressure in the control condition or during hydralazine treatment, but it did significantly ($p<0.01$) increase tail-cuff pressure during captopril treatment.

Estimated RFCs for each treatment are summarized in Figures 1 and 2. When the relationship of either urine volume or sodium excretion was plotted against tail-cuff pressure, compared with control values, hydralazine treatment produced a parallel shift of the RFC along the pressure axis, while captopril treatment produced a marked change in the slope of the RFC. When the reciprocal of the slope of the RFC was used as an index of the sensitivity of arterial pressure to steady state changes in sodium load, arterial pressure was found to be insensitive to dietary sodium during control or hydralazine conditions, while during captopril treatment, arterial pressure was altered significantly by increasing sodium intake (Figure 3).

### Table 3. Effect of Chronic (2 wk) Administration of Captopril or Hydralazine in 10 Adult SHR Fed High Salt Diet (Drinking 1% NaCl Ad Libitum)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Captopril (100 mg/kg)</th>
<th>Hydralazine (15 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/24 hr)</td>
<td>21.0±0.4</td>
<td>24.5±0.8*</td>
<td>22.0±0.6*†</td>
</tr>
<tr>
<td>Fluid intake (ml/24 hr)</td>
<td>93.0±7.8</td>
<td>74.1±4.8*</td>
<td>90.1±6.8*†</td>
</tr>
<tr>
<td>Urine volume (ml/24 hr)</td>
<td>60.8±6.5</td>
<td>42.0±4.8*</td>
<td>50.4±5.8*†</td>
</tr>
<tr>
<td>Sodium excretion (mmol/24 hr)</td>
<td>18.75±1.66</td>
<td>10.81±0.94*</td>
<td>14.68±1.32*†</td>
</tr>
<tr>
<td>Tail-cuff pressure (mm Hg)</td>
<td>220±5</td>
<td>210±4*</td>
<td>162±4*†</td>
</tr>
</tbody>
</table>

Values are means ± SE of average values measured twice during the second week of each cycle (see Table 1). *$p<0.05$, compared with control values (by Newman-Keuls test after repeated-measures ANOVA). †$p<0.05$, compared with captopril values (by Newman-Keuls test after repeated-measures ANOVA).
Discussion

We have used relatively simple experimental techniques to demonstrate that hydralazine and captopril produce characteristically different effects on the RFC of adult SHR. The estimated RFCs produced in the present study by using measurements of tail-cuff pressure and 24-hour urine collections agree well with published RFCs obtained with carefully controlled loading conditions and direct measurements of mean arterial pressure. For example, Norman et al.\(^5\) showed that the RFC in adult SHR was nearly vertical over the range of sodium intakes used in the present study, and other investigators have shown the relative insensitivity of arterial pressure in adult SHR to changes in dietary sodium.\(^{12,13}\) In addition, captopril is known to decrease the slope of the RFC in normotensive dogs,\(^{14}\) and the effect of captopril in increasing the sensitivity of arterial pressure to dietary sodium has been reported in SHR during the development of hypertension.\(^{19}\) Finally, the parallel shift of the RFC produced by hydralazine in this study agrees with the effect of this drug on the RFC of rats with established deoxycorticosterone acetate-salt hypertension.\(^{16}\) Thus, our results provide reasonable estimates of the RFC in untreated SHR and in SHR treated with antihypertensive drugs. If we assume that renal function is an important determinant of the steady state level of arterial pressure,\(^{1-3}\) these results provide useful clues to the mechanism of action of these agents used to treat hypertension.

Both hydralazine\(^6\) and captopril\(^9\) are effective antihypertensive drugs when given chronically in the SHR. Presumably, hydralazine acts primarily as a nonspecific peripheral vasodilator.\(^{15,18}\) The parallel shift of the RFC in hydralazine-treated SHR is consistent with predicted effects of a vasodilator (including renal vasodilation) in conditions where the hypertension is characterized by a vertical RFC.\(^{19}\) The primary physiological change leading to a sustained decrease in arterial pressure during chronic administration of converting enzyme inhibitors is less well understood, although peripheral vasodilation is frequently cited as the mechanism of action of these drugs.\(^{20,21}\) The results of Koike et al.,\(^{22}\) showing decreased regional vascular resistance in SHR treated chronically with captopril, seem to support this conclusion. In recent years, the effectiveness of captopril in decreasing peripheral vascular resistance and arterial pressure has been interpreted as evidence for an involvement of an abnormal vascular wall renin-angiotensin system in the maintenance of hypertension in SHR.\(^{23}\) Support for this hypothesis comes from studies showing differences in the renin-angiotensin system found in vascular tissue of SHR compared with that seen in normotensive rats\(^{24,25}\) and the correlation between the hypotensive effects of converting enzyme inhibitors and inhibition of the enzyme in various tissues, including blood vessels, during chronic treatment in SHR.\(^{26}\) Thus, inhibition of an abnormal vascular wall renin-angiotensin system has been suggested to lead to peripheral vasodilation and to explain the antihypertensive activity of captopril in renin-independent models of hypertension.\(^{23}\) If this were the case, then one might predict that hydralazine, a known vasodilator, and captopril would produce similar effects on the RFC. That this was not the case suggests that captopril and hydralazine have different effects on the mechanisms that contribute to the long-term control of arterial pressure.

Norman et al.\(^3\) have shown that the RFC of adult SHR is similar in shape to that seen in normotensive rats, but shifted along the pressure axis. Our results, showing a decreased slope of the RFC during captopril treatment in the SHR, support the conclusion that the renal effects of angiotensin II are important in maintaining the steep relationship between arterial pressure and sodium excretion, thus making arterial pressure relatively insensitive to changes in sodium intake.\(^{14}\) Based on these findings, we suggest that the major antihypertensive effect of captopril administered chronically in the adult SHR is due to removal of the renal effects of angiotensin II. This view supports the suggestion that the intrarenal renin-angiotensin system is an important site of action of converting enzyme inhibitors for producing chronic reductions in arterial pressure in SHR,\(^{26}\) although removal of the effect of circulating angiotensin II may also be involved. Captopril may decrease peripheral vascular resistance by inhibiting the vascular wall renin-angiotensin system in addition to altering renal function, but the changes in regional vascular resistance may also be secondary to local functional or structural adjustments associated with the hemodynamics of altered renal function. Vasodilation in the kidney may play a part in the specific action of captopril,\(^{24,21}\) but the overall effect on the RFC is quite dissimilar to that produced by a peripheral vasodilator such as hydralazine.
Moreover, the hypotensive effect of captopril may not be specific for hypertensive conditions, but may simply reveal the effect of chronic inhibition of the renal effects of angiotensin II, which are normally involved in the long-term control of arterial pressure.\textsuperscript{14, 27, 28} According to this reasoning, the fact that captopril lowers arterial pressure in the SHR does not necessarily mean that there are abnormalities in either the circulating or local tissue renin-angiotensin systems but rather that, even when the renin-angiotensin system is functioning normally, elimination of its important renal effects will lead to a decrease in arterial pressure at normal or low levels of sodium intake. This conclusion is supported by the observation that captopril lowers arterial pressure in normotensive animals\textsuperscript{14, 29} and by the studies of MacGregor et al., using normotensive and hypertensive subjects.

In summary, we have described a simple technique for estimating RFCs in SHR and have shown the differential modification of this relationship by two commonly used antihypertensive drugs. The results are consistent with the conclusion that the antihypertensive effect of captopril given chronically in the SHR is due primarily to inhibition of the normal renal effects of angiotensin II with subsequent changes in renal function, rather than to a generalized vasodilatation produced by inhibition of the vascular wall renin-angiotensin system.

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