Differential Pressor and Renal Vascular Reactivity to Angiotensin II in Spontaneously Hypertensive and Wistar-Kyoto Rats

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SUMMARY The suggestion has been made that the Okamoto strain of spontaneously hypertensive rats (SHR) shares some features with a subgroup of patients with essential hypertension, called nonmodulators. One feature of nonmodulators is a renal blood flow response to angiotensin II (ANG II) that is blunted on a high salt diet; the blunted renal vascular response is corrected by converting enzyme inhibition. Renal blood flow (electromagnetic flowmeter) and pressor responses to graded ANG II doses (5–300 ng) were assessed in 24 SHR and 24 Wistar-Kyoto rats (WKY) ingesting 1.6% Na. In comparison to WKY, blood pressure was higher in SHR (155 ± 4 vs 106 ± 2 mm Hg; p<0.001), renal blood flow was lower (6.9 ± 0.5 vs 8.2 ± 0.4 ml/min/g; p<0.05), and the pressor response to ANG II was enhanced, (p<0.0005) but the renal vascular response was blunted (p<0.005). Captopril (1–30 mg/kg) reduced blood pressure more in SHR than in WKY but increased renal blood flow similarly in both strains. The blunted renal vascular response to ANG II in SHR was reversed by captopril, but inhibition of converting enzyme in the kidney did not parallel systemic inhibition. Maximum blockade of converting enzyme in the kidney appears to require a larger captopril dose than is required for systemic inhibition. These results suggest that the renal blood supply in SHR also shares some of the characteristics of nonmodulators and that the action of captopril on the renal blood flow probably reflects reversal of inappropriate intrarenal ANG II formation. (Hypertension 9: 591–597, 1987)

KEY WORDS • sodium • angiotensin I • angiotensin II • converting enzyme inhibition • renal blood flow • captopril

AMONG the lines of evidence implicating the kidney in the pathogenesis of hypertension a particularly compelling observation involves the results of renal transplantation. In three unrelated strains of rats programmed genetically to develop hypertension, transplantation of a kidney from a hypertensive donor to a normotensive recipient raises the recipient's blood pressure.1-3 Conversely, removal of the kidneys from a hypertensive rat and replacement with a kidney from a normotensive donor prevents or reverses hypertension. Studies in humans are necessarily less direct, but two clinical studies on transplanted kidneys confirm the studies in rats.4,5 The genetic information leading to hypertension is clearly expressed in the kidney. Precisely what the abnormality in the kidney is that leads to hypertension remains unclear, and probably varies from model to model.

Among candidates for a pathogenetic renal abnormality, the renin-angiotensin system provides an attractive possibility. Interest in a contribution of the renin-angiotensin system to the pathogenesis of hypertension has generally focused on systemic actions of angiotensin II (ANG II), although direct pressor action of this system clearly is not responsible for the hypertension in any of the genetic models cited.1-3 Evidence has been accumulating, however, that an intrarenal action of this system contributes to the normal control of kidney function4 and may participate in the renal abnormalities in a subgroup of patients with essential hypertension called nonmodulators.7-9 Nonmodulators are a distinct group of patients with essential hyperten-
sion who have a strong family history of hypertension\(^8\) and an abnormal response of the adrenal and the renal blood supply to ANG II\(^10\) that is corrected by converting enzyme inhibition.

Spontaneously hypertensive rats (SHR) have a similar blunted adrenal response to ANG II on a low salt diet\(^11\) and are one of the strains in which hypertension "follows" the kidney after renal transplantation.\(^3\) In this study, we assessed the determinants of renal vascular reactivity to ANG II in SHR and normotensive Wistar-Kyoto rats (WKY) when in balance on a high salt diet, the diet that brings out the renal vascular abnormality in essential hypertension.\(^7\)\(^-\)\(^11\) Our initial finding, that SHR have a potentiated pressor response to ANG II but a blunted renal vascular response, led us to examine the influence of captopril on responsiveness, as converting enzyme inhibition corrects the renal vascular abnormality in nonmodulating essential hypertension.\(^7\)\(^-\)\(^8\)

**Materials and Methods**

The experiments were done on SHR and WKY obtained from Charles River Laboratories (Wilmington, MA, USA). The rats arrived at our animal facility when they were 9 weeks old and were fed a 1.6% Na diet obtained from Purina Test Diets (Richmond, IN, USA) for 2 to 4 weeks. The experiments were performed when the animals were 11 to 13 weeks old. Tap water was the drinking fluid with a sodium content of 0.5 to 1 mEq/L.

The day of the experiment the rat was anesthetized with pentobarbital sodium (15 mg/kg i.p., the dose adjusted to 75 mg/kg after weighing), supplemented as necessary at doses of 5 mg i.v. or i.p. during the experiment. The rat was weighed, shaved, and placed under a heat lamp to maintain a rectal temperature of 37 to 38°C. The ideal placement of the lamp was under a heat lamp to maintain a rectal temperature of 37 to 38°C. The ideal placement of the lamp was determined with a square wave blood flowmeter (Carolina Medical Electronics). Blood pressure and flow were measured with a square wave blood flowmeter (Carolina Medical Electronics). Blood pressure and flow were measured on a Grass 7 polygraph recorder (Quincy, MA, USA).

At the end of the experiment, the mechanical zero for blood flow measurement was determined by occluding the renal artery distal to the probe, the rat was killed with pentobarbital administered intravenously, a sample of urine for the determination of the sodium and potassium content was aspirated from the bladder, and the kidney and the heart were weighed. To estimate blood loss, the weight of all the cotton-tip applicators used to clean the operating field was determined soon after operation and during the equilibration period 5% dextrose in water equal to the estimated blood loss was infused. Further bleeding during the experiments was rare.

The agents employed (ANG II, NE, ANG I, captopril, and pentobarbital, all dissolved in 5% dextrose in water) were injected with calibrated micrometer syringes (Gilmore Instruments, Great Neck, NY, USA). ANG II and ANG I were obtained from Sigma (St. Louis, MO, USA), captopril from the Squibb Institute for Medical Research (Princeton, NJ, USA) and NE from Winthrop Breon Laboratories (New York, NY, USA).

Three sets of experiments were performed. The first set was performed on 32 animals (16 WKY and 16 SHR). In these animals doses of 5, 10, 30, 100, and 300 ng of ANG II and 100 ng of NE were injected at 5-minute intervals, beginning after a 20-minute postoperative equilibration period. The same procedure was repeated 20 minutes after the administration of captopril, 1 mg/kg i.v., in 16 rats (8 of each strain).

For the second set of experiments we measured the blood pressure and renal blood flow responses to eight SHR and eight WKY to 30 and 100 ng of ANG II, to 300 ng of NE, and to ANG I before and after the i.v. injections of cumulative captopril doses of 1, 3, 10, and 30 mg/kg. To determine the extent of converting enzyme inhibition, ANG I was injected at doses of 100 and 300 ng at the beginning and 300 ng thereafter. At the highest dose of captopril (30 mg/kg), ANG I was injected until the initial blood pressure response to 300 ng of ANG I was replicated.

The third set of experiments was designed as a time control to test the effect of time and anesthesia on blood pressure and renal blood flow responses to ANG II in five SHR. The protocol employed was the same as in the first set, but pentobarbital, 5 mg i.v., was injected instead of captopril.

Data from 36 WKY and 43 SHR were discarded without analysis, either for death related to anesthesia or operation, for excessive bleeding, or for instability of renal blood flow. Data were never discarded if a rat completed the experimental protocol.

Student's \( t \) test for unpaired data was used for the baseline comparisons in the two strains of rats. Chi square (with Yates correction) was employed to assess overall dose-response relationships for ANG I, ANG II, and NE, with symmetry around the median response as the index. The Fisher exact test was used to assess the responses to individual doses, since many fewer data points were involved. The null hypothesis was rejected when a \( p \) level less than 0.05 was found.
except in the case of multiple comparisons employed to assess the influence of individual captopril doses, when a p level less than 0.01 was accepted; four comparisons were involved.

Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Results

There was no difference in body or renal weight between SHR and WKY, although SHR showed the anticipated increase in cardiac mass (p<0.02; Table 1). Baseline urine sodium and potassium concentrations, the duration of the experiment, and the volume of fluid replacement administered were essentially identical in SHR and WKY, although the pentobarbital dose required to sustain surgical anesthesia was significantly greater in the SHR (p<0.001; see Table 1). Arterial blood pressure, as anticipated, was significantly higher in the SHR (p<0.001), but renal blood flow was lower (p<0.05).

SHR displayed the anticipated increase in the pressor response to graded doses of ANG II (p<0.0005) and, despite that, a directionally opposite, blunted response of the renal blood supply to ANG II (p<0.005; Figure 1). For the pressor response the difference between SHR and WKY became progressively larger with increasing ANG II dose, whereas the renal vascular responses to ANG II were approximately parallel over the entire range of ANG II doses employed (see Figure 1).

Captopril, in a dose of 1.0 mg/kg infused intravenously, induced the anticipated, prompt reduction in both the pressor and renal vascular response to ANG I (Figure 2). That dose reduced the pressor response to ANG I (300 ng) in SHR from 56±3 to 14±2 mm Hg, and increasing captopril doses produced no further blunting of the response to ANG I. Similarly, the blunting of the renal vascular response to exogenous ANG I induced by captopril peaked at a captopril dose of 1.0 mg/kg, and increasing captopril doses did not further blunt responses to ANG I. Baseline responses to ANG I did not differ significantly between the eight pairs of SHR and WKY, although the directional difference—a somewhat larger pressor and reduced renal vascular response in SHR—resembled the response to ANG II. Not anticipated was the observation that captopril was significantly less effective in blocking the pressor (p<0.001) and the renal (p<0.005) vascular responses to ANG I in SHR than in WKY (see Figure 2).

Captopril in a dose of 1.0 mg/kg enhanced the pressor response to ANG II in both SHR and WKY (Figure 3), but the result was an exaggeration of the difference between SHR and WKY, as the response to ANG II in SHR was enhanced significantly more (p<0.005). Similarly, captopril (1.0 mg/kg) enhanced the renal vascular response to ANG II significantly (p<0.025) in both SHR and WKY. Again, in the case of the renal blood supply, the enhancement of response was greater in the SHR; thus, the baseline difference in response to ANG II between SHR and WKY was no longer evident (0.6>p>0.5; see Figure 3).

Although the influence of captopril on pressor and renal vascular responses to exogenous ANG I reached a plateau at a captopril dose of 1.0 mg/kg, increasing captopril doses did not further blunt responses to ANG I. Baseline responses to ANG I did not differ significantly between the eight pairs of SHR and WKY, although the directional difference—a somewhat larger pressor and reduced renal vascular response in SHR—resembled the response to ANG II. Not anticipated was the observation that captopril was significantly less effective in blocking the pressor (p<0.001) and the renal (p<0.005) vascular responses to ANG I in SHR than in WKY (see Figure 2).

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Although the influence of captopril on pressor and renal vascular responses to exogenous ANG I reached a plateau at a captopril dose of 1.0 mg/kg (see Figure 2), increasing captopril doses had a further influence on both baseline blood pressure and renal blood flow (Figure 4). For that reason, responses to ANG II of the renal blood supply (Figure 5) and blood pressure (Figure 6) were assessed in WKY and SHR over the full range of captopril doses. The renal vascular response to ANG II was enhanced further in both WKY and SHR by increasing captopril doses, but the impact was progressively larger in SHR, so that the renal vascular response to ANG II was substantially larger (p<0.0005; chi square = 30.4) in SHR than in WKY.

### Table 1. Baseline Data in 24 WKY and 24 SHR

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKY</th>
<th>SHR</th>
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</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>244.3±4.9</td>
<td>251.6±2.6</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.97±0.03</td>
<td>1.06±0.02*</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>0.93±0.02</td>
<td>0.94±0.01</td>
</tr>
<tr>
<td>Pentobarbital dose (mg/kg)</td>
<td>95.7±3.2</td>
<td>142.9±6.0†</td>
</tr>
<tr>
<td>Duration of experiment (min)</td>
<td>190±11</td>
<td>193±12</td>
</tr>
<tr>
<td>Volume infused (ml)</td>
<td>1.1±0.2</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>Urine sodium (mEq/L)</td>
<td>125.9±12.6</td>
<td>124.9±16.0</td>
</tr>
<tr>
<td>Urine potassium (mEq/L)</td>
<td>123.4±9.1</td>
<td>104.5±8.7</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>106±2</td>
<td>155±4†</td>
</tr>
<tr>
<td>Renal blood flow (ml/min/g)</td>
<td>8.2±0.4</td>
<td>6.9±0.5†</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*p<0.02, **p<0.001, †p<0.05, compared with values for WKY.
with the three highest captopril doses employed. The difference between renal vascular responsiveness to ANG II in the SHR and WKY became significant at a captopril dose of 3 mg/kg (p < 0.01, Fisher exact test) and was larger at 30 mg/kg (p < 0.0005). Since the responses to ANG II at captopril doses of 3, 10, and 30 mg/kg were essentially identical (see Figure 5), it seems likely that the full efficacy of captopril had been identified. Captopril over that dose range induced a progressive enhancement of pressor responses to ANG II in SHR and WKY, but increasing captopril doses did not modify the difference between SHR and WKY (see Figure 6).
Because both baseline blood pressure and renal blood flow differed between the SHR and WKY, an additional calculation of percentage change in each index was made. The percentage change in renal blood flow was substantially larger in SHR when assessed with captopril doses of 3 to 30 mg/kg (p < 0.0001), but the percentage increase in blood pressure did not differ significantly between SHR and WKY.

Captopril did not modify pressor or renal vascular responses to NE (Table 2), which did not differ significantly in SHR and WKY. The time control group did not show a spontaneous shift in responsiveness to ANG II (Figure 7), despite the use of supplemental doses of pentobarbital in SHR.

**Discussion**

Our premise in this study was that SHR and non-modulators, a subgroup of patients with essential hypertension, share an abnormality in the angiotensin-mediated control of their renal blood supply. SHR are known to share two features with non-modulators, a strong family history of hypertension and a blunted adrenal response to ANG II that is most evident on a low sodium intake. In this study we showed a potentiated pressor and blunted renal vascular response to ANG II in SHR when in balance on a high sodium intake, the setting in which the renal vascular abnormality is evident in non-modulators. Converting enzyme inhibition with captopril potentiated the pressor response in both SHR and WKY, but even very large captopril doses did not alter the difference between SHR and WKY: the pressor response to ANG II in SHR remained substantially larger.

The renal blood supply, on the other hand, showed a different response to captopril. Captopril potentiated the renal vascular response to ANG II substantially more in SHR than in WKY, reversing the relative unresponsiveness evident at baseline. The blunted response to ANG II, characteristic of SHR, disappeared with captopril administration. The implications of this observation and the observation that the potentiation by captopril of renal vascular responses to ANG II occurred with captopril doses substantially higher than the dose required to obtain a maximal inhibition of converting enzyme, as judged from the response to exogenous ANG I, require discussion.

Many studies on smooth muscle, including vascular smooth muscle, have provided strong evidence for the mechanism by which captopril may enhance vascular responses to ANG II. Wherever studies on the angiotensin receptor on smooth muscle have been undertaken, a reciprocal relationship between ambient angiotensin concentration and the available number of angiotensin receptors has been found. One possible interpretation of the reduced renal vascular response to ANG II at baseline and of the impact of captopril on renal vascular responsiveness to ANG II in SHR, therefore, would be that the intrarenal ANG II concentration was higher in SHR than in WKY, accounting for their blunted response to angiotensin at baseline and, perhaps, their lower renal blood flow.

This interpretation raises two questions: first, how specific is the action of captopril likely to have been? Second, why did the dose-response relationship between captopril and exogenous ANG I not parallel the relationship between captopril dose and the renal vascular response to ANG II?

Many studies have shown an impact of captopril on prostaglandin formation and bradykinin degradation. Both prostaglandins and kinins have well-documented actions on renal vascular responses to ANG II. In both cases, increased prostaglandin formation and reduced bradykinin degradation, one would anticipate that captopril would further blunt the renal vascular response to ANG II, rather than enhance the response. On that basis, the simplest explanation for captopril's action is that the enhancement reflects a reduction in the intrarenal concentration of ANG II, although stronger evidence of such specificity is required.

A similar interpretation was given to the impact of converting enzyme inhibition on renal blood flow and its response to ANG II in some patients with essential hypertension—the non-modulators. In those patients, however, the converting enzyme inhibitor induced a larger increase in renal blood flow than that of pentobarbital in SHR.

**TABLE 2. Pressor and Renal Vascular Responses to Norepinephrine (100 ng)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal</th>
<th>After captopril</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BP responses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>+6.1 ± 0.3 (16)</td>
<td>+9.3 ± 2.4 (8)</td>
</tr>
<tr>
<td>SHR</td>
<td>+8.2 ± 1.4 (16)</td>
<td>+8.4 ± 0.5 (8)</td>
</tr>
<tr>
<td><strong>RBF responses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ml/min/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>−0.2 ± 0.1 (16)</td>
<td>−0.4 ± 0.1 (8)</td>
</tr>
<tr>
<td>SHR</td>
<td>−0.2 ± 0.1 (16)</td>
<td>−0.3 ± 0.1 (8)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Values in parentheses indicate the number of rats. There were no significant differences between SHR and WKY before or captopril (1 mg/kg i.v.). BP = blood pressure; RBF = renal blood flow.

**FIGURE 7. Reproducibility of responses to ANG II in five SHR when pentobarbital was used in place of captopril as a time control.** Note that neither pressor nor renal vascular responses to ANG II were influenced. See Figure 2 for key to abbreviations.
seen in modulators. In this study, renal blood flow did not increase more in SHR than it did in WKY. One possible explanation for the difference is the striking reduction in arterial blood pressure induced by higher doses of captopril in SHR, which may have limited the increase in renal blood flow.

Given the potentiated baseline pressor response to ANG II in the SHR, it seems unlikely that the very large fall in blood pressure induced by captopril reflects a reversal of ANG II–mediated hypertension. Perhaps the simplest explanation would be that much of the captopril-induced fall in blood pressure in SHR reflects mechanisms unrelated to ANG II formation, as has been reviewed recently. Such an interpretation would indicate that the determinants of response in the peripheral vessels and in the renal blood supply are fundamentally different in SHR than they are in the internal control group, as already indicated by the primary observations, which revealed a potentiated pressor but blunted renal vascular response to ANG II in SHR. Folkow et al. have argued that the enhanced pressor response in SHR reflects a change in the ratio of vessel wall radius to lumen diameter, increasing the mechanical advantage of smooth muscle contraction. The possibility that this phenomenon accounts for the enhanced pressor response to ANG II in SHR is supported by the observation in this study that the pressor response to ANG II in SHR was not parallel to that in WKY. The threshold response was similar in SHR and WKY, and potentiation in SHR became progressively larger with increasing angiotensin dose. For the renal vascular response to ANG II, however, the relationships in SHR and WKY were approximately parallel. Whatever differences existed in renal arteriolar geometry favoring an enhanced response in SHR were overcome by other factors under the conditions of our study. One would anticipate that a reduction in receptor number, for example, would induce a parallel shift in the dose-response relationship.

Presumably, the ANG I–induced increase in blood pressure and reduction in renal blood flow after intravenous injection of ANG I largely reflected conversion of ANG I to ANG II in the lung. Captopril in a dose of 1 mg/kg was adequate to reduce both responses to ANG I to a minimum, not exceeded by increasing captopril doses. If captopril indeed modified renal vascular responsiveness to ANG II through an impact on ANG II formation, it seems likely that the converting enzyme responsible lies within the kidney and not in the lung. Certainly, multiple lines of evidence indicate that converting enzyme exists at the vascular pole of the renal glomerulus and that ANG II is formed locally, probably participating in the tubuloglomerular feedback loop. Apparently, no data are available on the relation between captopril dose and blunting of tubuloglomerular feedback. The results of our study raise the possibility that earlier studies employed converting enzyme inhibitor doses that were too small to achieve full blockade.

Under the conditions of this study we found that renal blood flow was lower in SHR than in the control WKY. The renal blood flow in SHR generally has been found to be comparable to that in WKY in studies in which a standard rat diet was employed—one likely to be lower in sodium content than the diet used in this study. The renal blood flow we measured with the electromagnetic flowmeter in WKY was substantially higher than that reported in other studies, perhaps reflecting the impact of the high sodium intake.

A greater pressor response to ANG II on a high sodium diet in SHR confirms the reports of Ashida et al. The potentiation of pressor and renal vascular responses to ANG II in SHR after converting enzyme inhibition is also a confirmation of results reported by Richer et al. but they did not use WKY as internal controls. Our results are consistent with a special action of captopril on the kidney in SHR. The control of the renal blood supply in SHR differs not only from that of other systemic vascular beds but also from that in WKY, the best available genetic control group. The simplest explanation, that local ANG II formation is abnormal in SHR, raises the possibility of explaining the intrarenal abnormality that is transplanted when a kidney from SHR provokes hypertension in a normotensive host. It also lends itself to hypotheses that can be tested by new experiments. Although converting enzyme inhibitors show some pharmacological heterogeneity,29–31 renal vasodilator responses to converting enzyme inhibitors, including captopril, were not blunted by either aprotinin or indomethacin and probably do reflect their influence on ANG II formation in the kidney.

This investigation also lends support to the possibility, raised by earlier studies on adrenal release of aldosterone,11 that the SHR, at least in part, share an abnormality documented in some patients with essential hypertension. Should that be the case, studies in SHR would provide additional insights into renal mechanisms involved in the pathogenesis in at least some patients with essential hypertension.

Acknowledgments

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References


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