Vascular Wall Renin in Spontaneously Hypertensive Rats
Potential Relevance to Hypertension Maintenance and Antihypertensive Effect of Captopril

MAGDI M. ASAAD and MICHAEL J. ANTONACCIO, PH.D.

SUMMARY Relationships among systolic blood pressure (SBP), plasma renin activity (PRA), arterial renin concentrations (ARC), and venous renin concentrations (VRC) were examined in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats before and after treatment with captopril. The ARC was elevated in SHR relative to WKY whereas VRC was not. Similarly, ARC was related to SBP (r = 0.69, p < 0.01) whereas PRA was not (r = 0.04). Captopril (100 mg/kg daily by mouth for 8 days) decreased blood pressure significantly in both SHR and WKY. PRA as well as ARC and VRC were all increased by captopril. Bilateral nephrectomy virtually eliminated PRA but ARC was not significantly reduced over a 24-hour period. Bilateral nephrectomy also markedly attenuated the acute antihypertensive effects of captopril in SHR; however, a modest effect was still apparent. It is suggested that ARC in SHR, being higher than in WKY, may play a role in the genesis or maintenance of hypertension in this model. Furthermore, the effects of captopril in both intact and nephrectomized SHR may be related to the ability of captopril to inhibit the vascular formation of angiotensin II. Finally, vascular renin is probably not renal in origin and responds to typical feedback inhibition as unmasked by captopril administration.

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KEY WORDS • vascular renin • SHR • antihypertensive • captopril • angiotensin

In models of hypertension clearly associated with high plasma renin activity (PRA) such as acute two-kidney renal hypertension, agents that interfere with the formation of, or receptor activation by, angiotensin II (AngII) are very effective in reducing blood pressure. Thus, renin inhibitors,1,2 angiotensin-converting enzyme (ACE) inhibitors,3-8 and AngII antagonists9 have all been shown to either prevent the development of two-kidney renal hypertension or reduce blood pressure shortly after the development of this form of hypertension. In chronic two-kidney one clip hypertension as well as one-kidney one clip and genetic hypertension, however, the relationship between PRA in the maintenance of hypertension and the antihypertensive effectiveness of inhibitors of the renin-angiotensin system is no longer clear. Thus, AngII antibodies and receptor antagonists do not substantially reduce blood pressure in chronic one- or two-kidney one clip hypertension or in spontaneously hypertensive rats (SHR) which are models that have normal or low PRA.2,4,9,10 Renin and ACE inhibitors are clearly effective, however, in these normoreninemic hypertensive models.2,11-13 In particular, captopril, an orally active inhibitor of ACE, normalizes blood pressure and prevents the development of hypertension in adult SHR.13,14 Moreover, captopril is very effective in reducing blood pressure in both acute and chronic one and two-kidney one clip hypertensive dogs and rats.15 It is curious that captopril and other ACE inhibitors are dramatically effective in so-called "normal renin" hypertension whereas other agents that disrupt this system by other mechanisms are ineffective.

Recently, it has been argued that vascular renin or renin-like activity (hereafter referred to as vascular renin for simplicity), and its consequent generation of AngII, might be more important in certain forms of hypertension than PRA. Indeed, it was recently demonstrated15 that arterial wall renin concentration (ARC), but not venous wall renin concentration (VRC), was elevated in both SHR and chronic two-kidney one clip hypertension, but PRA was normal.15 The purpose of this study was to examine the effects of captopril on renin activity, both in plasma and vasculature, and on blood pressure in order to clarify the mechanism of the antihypertensive action of this agent in SHR.

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Methods

Blood Pressure Determinations

Twenty SHR and 20 WKY normotensive rats (Charles River Breeding Laboratories) were housed individually for at least 1 week and given food and water ad libitum. For the first part of the study, the SHR and WKY were randomly divided into four groups, and treated with 100 mg/kg captopril by gavage daily for 8 days. They were weighed daily and their SBP was recorded indirectly using the tail cuff procedure previously described. Immediately following the final SBP determination, the animals were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). For PRA, plasma sodium (Na⁺), potassium (K⁺), and protein determinations, 5 ml of blood was obtained without hemolysis by heart puncture in a syringe moistened with heparin (200 units/ml).

In other studies, approximately 18 days before each of the dose intervals described above, the mean arterial blood pressure (MAP) of SHR was recorded by indwelling abdominal aortic cannulas implanted according to the method of Weeks and Jones. After the 18-day recovery period, direct MAP of conscious rats was recorded by the method of Laffan et al. modified as follows: the signal from the transducer was digitized in a 10-list A/D converter with input to a PDP 11/05 computer. The program was designed to sense and store samples at a rate of 125/sec for each rat. These parameters were averaged and stored as the MAP. Data were obtained from each rat every 5 minutes for 8 hours; six such sets of data were averaged to give a mean value representing a 30-minute sample, and this 30-minute figure was stored for subsequent analysis. The data were transferred serially to a host computer (PDP 11/40) for final analysis.

Bilateral Nephrectomy and Sham Surgery of SHR

Under pentobarbital anesthesia (35 mg/kg i.p.), a group of SHR was nephrectomized by flank incision; the kidneys were decapsulated to avoid adrenalectomy, the renal pedicle ligated, and the kidneys removed. At 1, 2, 4, and 24 hours following surgery, blood samples and arterial tissues were obtained from all the animals as described below.

Vascular Tissue Preparation and Renin Determinations

In the anesthetized rats, a midline incision was made, and the major vessels (abdominal aorta and vena cava) with their branches were exposed by careful gentle dissection, trimmed of all connective tissues, and then opened longitudinally in situ. Immediately, the opened vessels were flushed with ice-cold saline, and traces of coagulated blood were removed manually. The vessels were then removed, washed in four successive ice-cold saline solutions containing EDTA (1.5 mg/ml), and blotted over filter paper between each wash. The blot-dried tissues were then weighed, frozen using a dry ice-acetone mixture, and stored below -70°C until used.

For renin determination, the vascular tissues were thawed, then cut into small pieces with a razor blade. The process of freezing and thawing was repeated several times. The tissues were then homogenized in ice-cold normal saline containing EDTA (1.5 mg/ml) with a motor-driven glass homogenizer fitted with a Teflon pestle. During the homogenization, the tissues were kept over ice at all times. Aliquots of the homogenate (10-20 mg wet tissue) were incubated with renin substrate (25 mg), tromethamine-malic acid buffer (pH 7.4), 8-hydroxyquinoline (1.7 mM), and dimercaprol (6.5 mM). Portions of this mixture were incubated at 37°C; others were kept at 4°C. Generated AI was radioimmunoassayed using the Squibb kit (Squibb Institute, Princeton, New Jersey). Vascular tissue renin concentration is expressed as ng AI/g wet tissue/hr. The PRA was determined by radioimmunoassay also utilizing the Squibb kit and expressed as ng AI/ml/hr.

Preparation of Renin Substrate

Renin substrate was prepared according to the procedure described by Boucher et al. When renin substrate concentrations equal to those used in this study were incubated with rat kidney tissue homogenate, the AI generated was approximately 10-fold higher than...
that formed by vascular tissue homogenate. Thus, the substrate concentration used was considered to be in excess.

Plasma Sodium, Potassium, and Protein Determination

Plasma Na+ and K+ concentrations were determined using the Flame Photometer 343 (Instrumentation Laboratory Inc., Cambridge, Massachusetts); plasma protein concentration was determined according to the procedure of Lowry et al.19

Results

Effect of Captopril on Systolic Blood Pressure

Captopril treatment (100 mg/kg p.o. daily for 8 days) resulted in a significant reduction of SBP in both conscious SHR (−38.2 ± 4.8 mm Hg, p < 0.001) as well as WKY (−18.4 ± 4.6 mm Hg, p < 0.01) (fig. 1).

Plasma Renin Activity and Vascular Renin Concentration

Table 1 summarizes the effects of captopril on PRA, VRC, and ARC. The PRA of untreated SHR and WKY was the same. After captopril treatment, the PRA was markedly elevated in both SHR and WKY, and slightly but not significantly more so in the SHR. The ARC of untreated SHR was significantly higher in comparison with the ARC of untreated WKY. In contrast, the VRC was similar in SHR and WKY. After captopril treatment, the ARC and VRC of both SHR and WKY were significantly elevated, the increase being greater in WKY.

The relationships between PRA, ARC, and SBP in both groups of SHR are shown in figure 2. It is apparent that there is no relationship between SBP and PRA (r = 0.04) but there is a significant correlation between SBP and ARC (r = 0.69, p < 0.01). In WKY, ARC was not correlated with PRA in either captopril-treated (r = −0.18) or untreated (r = 0.11) groups; similarly, VRC was not correlated with PRA in treated (r = 0.18) or untreated (r = −0.49) WKY. In contrast, the ARC of SHR was correlated with PRA in both captopril-treated (r = 0.81, p < 0.05) and untreated (r = 0.87, p < 0.05) rats. Similar to the results for WKY, the VRC of both treated (r = 0.44) and untreated (r = 0.45) SHR was not correlated with PRA.

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**Table 1. Effects of Captopril (100 mg/kg p.o. daily for 7 days) on Plasma Renin Activity (PRA), Arterial Renin Content (ARC), Venous Renin Content (VRC), and Plasma Sodium and Potassium Levels in Spontaneously Hypertensive and Wistar-Kyoto Normotensive Rats**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Spontaneously hypertensive rats</th>
<th>Normotensive rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 8)</td>
<td>Captopril (n = 10)</td>
</tr>
<tr>
<td>PRA (ng Al/ml/hr)</td>
<td>24.3 ± 4.6</td>
<td>106.6 ± 10.8*</td>
</tr>
<tr>
<td>ARC (ng Al/ml/hr)</td>
<td>70 ± 4.0</td>
<td>150 ± 14.4</td>
</tr>
<tr>
<td>VRC (ng Al/g/hr)</td>
<td>120.0 ± 18.3</td>
<td>279.7 ± 36.6</td>
</tr>
<tr>
<td>Na+ (mEq/liter)</td>
<td>143.3 ± 0.6</td>
<td>141.7 ± 0.3</td>
</tr>
<tr>
<td>K+ (mEq/liter)</td>
<td>4.7 ± 0.3</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>Plasma protein (g%)</td>
<td>5.5 ± 0.2</td>
<td>5.4 ± 0.2</td>
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</tbody>
</table>

* p < 0.01; control vs captopril-treated rats.
† p < 0.005; control vs captopril-treated rats.
‡ p < 0.01; control vs captopril-treated rats.
§ p < 0.05; spontaneously hypertensive vs normotensive rats.
|| p < 0.01; spontaneously hypertensive vs normotensive rats.

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**Figure 2. Relationships among plasma renin activity, arterial renin concentration, and systolic blood pressure of SHR and Wistar-Kyoto normotensive rats (NTR). Values shown are mean ± se of at least six determinations.**
Effect of Captopril on Plasma, Sodium, Potassium, and Protein Concentration

Plasma Na⁺, K⁺, and protein concentrations of SHR and WKY were not different from each other. In addition, captopril had no significant effect on plasma concentrations of Na⁺, K⁺, or protein in either SHR or WKY (table 1).

Effect of Bilateral Nephrectomy on Blood Pressure, Plasma Renin Activity, and Arterial Renin Concentration of SHR and the Antihypertensive Response to Captopril

The PRA declined dramatically between 1 and 24 hours after bilateral nephrectomy (Fig. 3) and was barely detectable 24 hours after nephrectomy. The PRA of sham-operated SHR, 1 hour, 2 hours and 4 hours after surgery was approximately two-fold higher than that after 24 hours, probably because of the effects of surgery. However, 1 hour, 2 hours and 4 hrs after nephrectomy, ARC of SHR did not differ significantly from that of the sham-operated animals. Twenty-four hours after nephrectomy in SHR the ARC was approximately 65% that of sham values.

In other groups of conscious SHR in which MAP was measured by direct aortic cannulation, the MAP was significantly elevated either 3.5 or 24 hours after bilateral nephrectomy, whereas sham nephrectomy showed no effect on MAP (table 2). Captopril (100 mg/kg p.o.) reduced MBP by 20, and 14 mm Hg at 3.5 and 24 hours respectively after bilateral nephrectomy, whereas vehicle alone caused a 4 mm reduction in MBP at the respective times (table 2). In sham-nephrectomized rats, captopril decreased MAP by 43 and 47 mm Hg at 3.5 and 24 hours after surgery (table 2). The differences in MAP between the vehicle- and captopril-treated groups were significantly different from each other (p < 0.05) at the 3.5 hour and 24-hour time periods following bilateral nephrectomy.

Discussion

The possibility of the presence of renin-like enzyme in artery extracts was first demonstrated by Dengler.20 Gould et al.21 further demonstrated that this enzyme hydrolyzed natural or synthetic renin substrate to form a vasopressor substance, had the same activation energy and pH optimum as renin, and was inhibited by antirenin antibody. The present study demonstrated that arterial — but not venous — wall renin is significantly higher in SHR than in their genetically comparable normotensive WKY counterparts despite a similar PRA in both groups. Furthermore, SBP was significantly correlated with ARC but not PRA, suggesting a possible cause and effect relationship. These data agree with those of others15,22 who have shown that adult, but not young, SHR had elevated ARC as measured by bioassay. Garst et al.16 also found that the ARC in either acute or chronic two-kidney one clip hypertension was significantly increased in comparison with normotensive controls. More important, PRA was normal in chronically hypertensive rats whereas arterial renin remained elevated, once again suggesting a maintenance role for arterial renin in hypertension. Similarly, Thurston et al.23 also reported elevated aortic renin in two-kidney one clip hypertension in rats and further demonstrated its long half-life in compari-
son with plasma renin. Basso et al. showed that renin-like activity of arterial walls in one-kidney one clip hypertensive rats was significantly higher than in one-kidney normotensive rats.

Our results are not in agreement, however, with those of Barrett et al., who found no increase in the ARC of SHR. In our study, the majority of arterial renin in SHR apparently was not derived from plasma renin since bilateral nephrectomy resulted in a rapid and marked decline of PRA whereas the ARC was only modestly reduced. Thus, this study and others show that a significant portion of vascular renin remains when plasma renin is either very low or not detectable, and suggests that this residual vascular renin activity is not due simply to adsorption of circulating renin onto vascular tissue. More important, cultured arterial smooth muscle cells derived from dog aorta produced AI when incubated with renin substrate at neutral pH. These cell cultures, furthermore, were immunoreactive to renin-specific antisera, producing strong evidence that vascular smooth muscle is itself capable of generating renin and forming AI locally. In this study, special care was taken to wash thoroughly and remove manually any adhered blood from vascular tissue so that any contamination from plasma was highly unlikely. Furthermore, residual AI (i.e., AI measured at 4°C incubations) was very low in vascular tissue despite relatively high levels of residual Al in plasma, especially in captopril-treated rats, a fact that also makes plasma contamination of vascular tissue unlikely. Several other studies have also demonstrated the presence of vascular renin 24 hours after nephrectomy in both normotensive and hypertensive animals. That the localization of renin either in plasma or vasculature could result in functionally important differences was first suggested by Daum et al. who found that infusion of aminopeptidase reduced the AI pressor response in rats but was less effective against the renin response. This implied that locally formed AI was less susceptible to aminopeptidase destruction. The localization of the majority of arterial renin in the adventitia (75%) of aorta supports such a hypothesis.

In the present study, special care was taken to eliminate the possibility of "pseudorenin" activity in vascular preparations: 1) the incubation mixture was maintained at a pH of 7.4, which prevents the activation of prorenin to active renin and also eliminates any significant acid protease or cathepsin-D activity since both these enzymes are inactive above pH 6.0. (Although it is understood that the pH optimum of renin is 6.5, we decided to use a pH of 7.4 since this is a more accurate reflection of the ability of renin to form AI in vivo). 2) The use of homologous renin substrate in this study (which was necessary since vascular tissue was found to be free of substrate) rather than the synthetic tetradecapeptide substrate eliminated the possibility of pseudorenin as a factor in the generation of AI in the system. 3) Inhibition of AI synthesis by captopril resulted in increased renin activity in vascular tissue, suggesting that the enzyme was under typical negative feedback inhibition by its final product.

Finally, we have recently shown that antibody to purified rat renal renin totally inhibits vascular renin activity at neutral pH, but is inactive at pH 5.0. Many lines of evidence suggest that the ability of inhibitors of the renin-angiotensin system to reduce blood pressure in various models of hypertension is related to their relative ability to reach the renin-angiotensin system of the vasculature. Thus, whereas AI antibodies are effective in decreasing blood pressure when PRA is high (acute one- and two-kidney one clip hypertension), they have little or no effect when PRA is normal or low, as in chronic one- and two-kidney one clip hypertension and spontaneous hypertension.

### Table 2
Effect of Sham or Actual Bilateral Nephrectomy (Nx) on Blood Pressure Responses to Either Captopril or Vehicle in SHR

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean blood pressure (mm Hg)</th>
<th>After dosing (p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before surgery</td>
<td>After surgery</td>
</tr>
<tr>
<td>Sham Nx</td>
<td>182 ± 5 (n = 7)</td>
<td>179 ± 3</td>
</tr>
<tr>
<td>Bilateral Nx (3.5 hrs)</td>
<td>182 ± 4 (n = 5)</td>
<td>205 ± 3 (p &lt; 0.01)</td>
</tr>
<tr>
<td>Bilateral Nx (3.5 hrs)</td>
<td>182 ± 4 (n = 5)</td>
<td>203 ± 6 (p &lt; 0.01)</td>
</tr>
<tr>
<td>Sham Nx (24 hrs)</td>
<td>186 ± 6 (n = 6)</td>
<td>184 ± 6 (p &lt; 0.01)</td>
</tr>
<tr>
<td>Bilateral Nx (24 hrs)</td>
<td>173 ± 3 (n = 9)</td>
<td>188 ± 5 (p &lt; 0.01)</td>
</tr>
<tr>
<td>Bilateral Nx (24 hrs)</td>
<td>178 ± 3 (n = 8)</td>
<td>192 ± 3 (p &lt; 0.05)</td>
</tr>
</tbody>
</table>
It has been suggested that the ineffectiveness of All antiseras to decrease blood pressure when PRA is low is due to poor penetration to intravascular rather than plasma sites of AI generation.14 This suggestion is also compatible with the observed antihypertensive effects of lipid soluble renin inhibitors in both acute and chronic one- and two-kidney one clip hypertension.3 Similarly, inhibition of the vascular renin-angiotensin system by the ACE inhibitor, captopril, is also consistent with its effectiveness in chronic one- and two-kidney one clip hypertension.42 Recently, we have demonstrated43 that captopril inhibits sympathetic function in SHR, an effect specific for the vasculature and linked to the vascular renin-angiotensin system. We suggested and offered evidence that the vascular renin-angiotensin system may be functional and important in both renal and spontaneous hypertension and that the different effectiveness of All antagonists and captopril under certain conditions may be explained in part by their relative abilities to inhibit this vascular system. This hypothesis is also consistent with the inability of captopril alone to either decrease or prevent hypertension caused by DOCA and salt, a very low renin model in both plasma and the vasculature.44, 45

In this and other studies,42 the antihypertensive effect of captopril as well as other ACE inhibitors was markedly reduced by prior nephrectomy. Captopril still had a modest antihypertensive action in nephrectomized SHR, however, an effect apparently unrelated to PRA which was essentially unmeasurable in these rats. Moreover, the residual antihypertensive effect of captopril in nephrectomized SHR could not be due only to an inhibition of plasma AI generation since All antiseras and receptor antagonists are ineffective even in intact SHR (see Introduction). In addition, Thurston and Swales46 have shown that renin-induced hypertension in nephrectomized hypertensive rats was reduced to normal by SQ 20,881, showing that the pressor response to renin was maintained by continuous AI generation, probably vascular. Saralasin pretreatment prevented the effects of SQ 20,881, showing that the pressor response to renin was maintained by continuous AI generation, probably vascular. Saralasin treatment prevented the effects of SQ 20,881, suggesting that kinins were not involved. Captopril also has a significant antihypertensive effect in bilaterally nephrectomized chronic two-kidney, one-clip renal hypertensive rats although, as in SHR, of shorter duration and magnitude than in intact animals.47

The present study provides evidence that arterial renin is elevated in SHR, is probably not derived in toto from the kidney, responds to negative feedback inhibition, and is of potential importance in the maintenance of hypertension and antihypertensive effectiveness of captopril in this model. Other possibilities for the antihypertensive mechanism of captopril have not been ruled out by these experiments, however. For instance, the possibility of both kinin and prostaglandin involvement have been demonstrated and should be taken into account.48-51

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