Hypotensive Action of Captopril in Spontaneously Hypertensive and Normotensive Rats
Interference with Neurogenic Vasoconstriction

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SUMMARY The effects of captopril and angiotensin II on adrenergic neurotransmission have been studied in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). In a pithed rat preparation, vasoconstrictor responses evoked by spinal stimulation were greater in SHR than WKY (p < 0.01). Captopril reduced responses to electrical stimulation and this reduction was greater in the SHR (p < 0.001). Bilateral nephrectomy reduced the vasoconstrictor responses to nerve stimulation in both strains of rat and abolished the effects of captopril. In an isolated perfused mesenteric artery preparation, responses to nerve stimulation in the absence of angiotensin II were greater in SHR than WKY (p < 0.05). Angiotensin II potentiated responses from both strains of rat, however the amplitude of the potentiation was greater in preparations from the SHR than those from WKY (p < 0.002). Captopril (30 mg/kg by mouth) reduced blood pressure in conscious SHR over a 5-day dosing period. In WKY rats, no hypotensive action of captopril was observed. However, in another normotensive strain, the Alderley Park Wistar rat (APW), captopril lowered blood pressure. Plasma renin activity was not significantly different among these three strains of rat. The APW have previously been shown to be very sensitive to the adrenergic potentiating actions of angiotensin II. Captopril thus lowers blood pressure in SHR and APW, and both these strains are sensitive to the adrenergic potentiating actions of angiotensin II. It does not lower blood pressure in WKY, which is relatively insensitive to these actions of the octapeptide. Therefore, the hypotensive action of captopril in the rat may be due to its interference with the adrenergic potentiating effect of angiotensin II.

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KEY WORDS • angiotensin • converting enzyme inhibition • pithed rat • perfused rat mesenteric artery • sympathetic nervous system • adrenergic vasoconstriction • plasma renin activity • blood pressure • captopril

THE mechanism of action of captopril in lowering blood pressure is unclear. In both humans and animals, a fall in blood pressure is seen in those with elevated, normal, and low levels of circulating renin.1″ This could suggest that the inhibition of angiotensin II formation is not an important factor in the hypotensive action of captopril. However, recent studies suggest that the level of angiotensin II that participates in the control of blood pressure is closer to the normal range than previously realized.7′8 In addition, a functional interaction of the octapeptide with the sympathetic nervous system has been largely over-

looked. This is particularly important since in many forms of hypertension there is evidence of an over-activity of the sympathetic nerves.9′10

We have recently shown in the pithed rat11,12 and in rat isolated mesentric arteries13 that captopril interferes with adrenergic neurotransmission by an angiotensin dependent mechanism. Thus, converting enzyme inhibitors could lower blood pressure by attenuating neurogenic vasoconstriction. Since these observations were made in normotensive Alderley Park Wistar rats (APW), their relevance to the hypotensive action of captopril was unclear as it was not known whether captopril lowers the blood pressure of this strain.

In the present study we have extended our observations of the effects of captopril and angiotensin on adrenergic neurotransmission to the spontaneously hypertensive rat (SHR) in which the drug is known to lower blood pressure4′14 and to the normotensive Wis-
tar-Kyoto rat (WKY) in which it does not.4 The hypotensive properties of captopril have been assessed in these three strains of rat (APW, SHR, WKY), and plasma renin activity (PRA) measured.

Methods

Three strains of rats 4 to 6 months of age weighing 230 to 280 g were used: spontaneously hypertensive rats (SHR), Wistar-Kyoto rats (WKY), and Alderley Park Wistar rats (APW). Female rats were used in the majority of the experiments. Mesenteric artery preparations were taken from rats of both sexes; however, there were no sex-related differences in the responses of these arteries (see results).

Pithed Rat Preparation

Female SHR and WKY rats were anesthetized with halothane, their trachea cannulated, and the animals quickly pithed via the orbit.13 Artificial respiration with room air was immediately started using a Palmer pump. The pithing rod was insulated throughout its length apart from a section 5 cm from the tip. A carotid artery was cannulated for blood pressure measurement, and heart rate was derived from the pressure pulse. Drugs were injected via a jugular vein — atropine (1 mg/kg) and tubocurarine (1 mg/kg) — and the vagi sectioned to prevent any parasympathetic effects and voluntary muscle activity. The blood pressure of both strains of rat after pithing was the same. The blood pressure response was recorded during stimulation of the sympathetic outflow (lower thoracolumbar) at 1 to 30 Hz, 0.5 msec pulses at supramaximal voltage (60 V) for 10 seconds. Norepinephrine was given intravenously at doses of 0.04 to 2.0 μg/kg.

Control frequency and dose response curves were obtained in the groups of animals, allowing 2 to 5 minutes between responses. Captopril (1 mg/kg) was then given intravenously at 30 to 40 minutes after pithing, and the response curves were repeated starting 3 minutes after the bolus injection. In another group of SHR, saline (0.9% NaCl) was administered as a control and response curves were again repeated. In these animals, plasma renin activity (PRA) was also measured.

Separate groups of SHR and WKY were anesthetized with halothane, and flank incisions were made from which each kidney was exteriorized and removed after ligation of the renal artery, vein, and ureter. Care was taken not to interfere with surrounding tissues, in particular the adrenal glands. The wounds were closed with muscle suturing and skin autoclips. The pithing procedure was carried out as previously described, and frequency and dose response curves were obtained as in the rats with intact kidneys before and after captopril (1 mg/kg i.v.). In a further group of rats, a sham nephrectomy was performed using the same operative procedure as described above except that the kidneys were manipulated but not removed. Response curves before and after captopril (1 mg/kg i.v.) were measured in these rats.

Mesenteric Artery Preparation

Male and female SHR and WKY were anesthetized with pentobarbitone sodium (60 mg/kg i.p.). The abdomen was opened and the superior mesenteric artery cannulated. The artery and arterioles were removed and perfused with Krebs-Ringer solution at a constant flow of 6.5 ml/min, as previously described.12 The composition of the Krebs-Ringer perfusion fluid (mmole/liter) was NaCl, 118.2; KCl, 4.7; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 2.5 NaHCO3, 25; calcium disodium EDTA, 0.026; and glucose, 5.0; the solution was aerated with 5% CO2 in O2 and maintained at 37°C. Mesenteric artery preparations were allowed to equilibrate for 1 hour before the experiment was started. The adrenergic nerves supplying the arteries were electrically stimulated (2 msec pulse width, supramaximal voltage, 28–32 V, 20-second trains) via periarterial platinum electrodes. Electrical stimulation using these parameters has been shown previously to activate postganglionic adrenergic nerves in this preparation.16 Vasoconstrictor responses were recorded as an increase in perfusion pressure using a pressure transducer (Bell and Howell) and a chart recorder (Lectromed MX2).

Vasoconstrictor responses (~10 mm Hg) were evoked at 5-minute intervals by electrical stimulation (3–12 Hz). When three consecutive responses of constant amplitude were obtained, perfusion with angiotensin II was started. Three responses were obtained in the presence of each concentration of the peptide. Finally, the tissues were perfused with normal Krebs-Ringer solution until vasoconstrictor responses returned to a stable control level.

Full frequency-response curves were also constructed (1–16 Hz, 20-second train duration) in the absence and presence of angiotensin II (9.7 x 10-9M, 15-minute initial equilibration). Previous studies have shown that frequency response curves are stable and repeatable in this preparation.13 At the end of the experiment, a maximal response was evoked (24 Hz, 1-minute duration) to confirm that the potentiating effects of angiotensin II at the higher frequencies of stimulation (12–16 Hz) were not limited by the maximal vasoconstrictor response of the preparation.

Conscious Rat Preparation

Female rats (SHR, WKY, APW) were surgically implanted with vinyl catheters by the method of Popovic and Popovic17 under halothane anesthesia and allowed 24 hours for recovery. Aortic blood pressure was recorded directly via pressure transducer (Bell and Howell Type 4-422), and heart rate was electronically derived from the pressure pulse and displayed on a pen recorder (Lectromed MX2) together with blood pressure. The catheters were filled with saline (0.9% NaCl) containing heparin (50 IU/ml) when not in use.

During measurements, rats were placed in open-ended perspex tubes which allowed them to stand comfortably. Blood pressure and heart rate were recorded at intervals during the next 2 days for control values to be determined. Captopril dissolved in saline was then
dosed orally for 5 days at 30 mg/kg once daily. Blood pressure was measured each morning before dosing and again at 2, 5, and 24 hours after the doses on each of 5 days. PRA was measured in these rats in arterial blood taken the day before dosing commenced. Control groups of animals were subjected to the same procedure and saline vehicle (0.9% NaCl, 1 ml) was given orally for 5 days. Captopril (1 mg/kg) was also administered intravenously to conscious rats of all three strains, and the effects on blood pressure were measured as described above.

Measurement of Plasma Renin Activity

Estimations were made on 0.25 ml of arterial blood collected into chilled tubes containing 10 μl of 6% EDTA. PRA was then measured by the method of Haber et al.18 by radioimmunoassy of generated angiotensin I with modification as described previously.19

Statistical Methods

Statistical analysis of the data was by analysis of variance and by Students' paired or unpaired t test, as appropriate.

Results

Table 1 shows the control blood pressure, heart rate, and PRA measured in conscious rats of the three strains. The blood pressure of the SHR was significantly higher (p < 0.001) than that of the two normotensive strains. The PRAs were not significantly different among the three strains (analysis of variance) although the SHR tended to have higher levels of PRA than the normotensive strains. The heart rate of WKY was significantly lower than that of both SHR (p < 0.01) and APW (p < 0.001).

Effect of Captopril on Vasoconstrictor responses in Pithed SHR and WKY

Vasoconstrictor responses evoked by spinal stimulation were greater in SHR than WKY at all frequencies (p < 0.01) (fig. 1). Captopril (1 mg/kg i.v.) significantly attenuated the pressor responses evoked by nerve stimulation in both SHR and WKY, but the attenuation was greater in absolute terms at all frequencies in SHR (p < 0.001). Control responses to norepinephrine were similar in SHR and WKY and were reduced to a similar extent by captopril (fig. 1). Saline administered to SHR did not attenuate responses to either nerve stimulation or norepinephrine (fig. 2). PRA was high in the SHR immediately after pithing (38.5 ng Al/ml plasma/hr) and remained elevated throughout the duration of the experiment. At 2 to 3 hours after bilateral nephrectomy, which reduced PRA to unmeasurable levels, the vasoconstrictor responses of both SHR and WKY to nerve stimulation and norepinephrine were reduced when compared with control rats except at the lowest frequency in WKY and the lowest norepinephrine concentration in both strains of rats (p < 0.001). The effect of captopril was now abolished (fig. 3). In animals that had undergone a sham nephrectomy, captopril still significantly attenuated responses to both nerve stimulation and norepinephrine (fig. 2).

Effect of Angiotensin II on Responses Evoked by Sympathetic Nerve Stimulation in Perfused Mesenteric Arteries from SHR and WKY

Responses evoked by adrenergic nerve stimulation in the absence of exogenous angiotensin II were of greater amplitude in preparations from the SHR than WKY (p < 0.05) (fig. 4). To evoke responses of similar amplitude in mesenteric arteries from SHR (11.4 ± 1.0 mm Hg) and WKY rats (12.3 ± 1.4 mm Hg), a significantly lower frequency of stimulation was used in preparations from the hypertensive animals (SHR = 4.0 ± 0.3 Hz; WKY = 8.9 ± 0.8 Hz). A low concentration of angiotensin II (9.7 x 10⁻⁹ M) only significantly potentiated the amplitude of these responses in the SHR (fig. 5). Higher concentrations potentiated the responses in mesenteric arteries from both strains of rat. However, the amplitude of this potentiation was significantly greater in preparations from the SHR than those from the WKY strain (p < 0.002) (fig. 5). Angiotensin II (9.7 x 10⁻⁸ M) caused a significant increase in the amplitude of responses evoked by 4, 6, 8, and 12 Hz electrical stimulation in arteries from the SHR. However, the same concentration of angiotensin II had only minimal effects on the amplitude of responses in arteries from WKY rats (fig. 4), significant potentiation only occurring at 4 Hz. There was no significant difference between the responses of arteries from male and female rats (SHR or WKY) to nerve stimulation or to angiotensin II.

Effect of Captopril Administered for 5 Days to SHR, WKY, and APW

Captopril (30 mg/kg p.o.) reduced blood pressure significantly in SHR by 20 mm Hg on Day 1 and by 35 to 40 mm Hg by Day 3 (fig. 6). The maximal effect was observed 2 hours after dosing on these days. There

### Table 1. Resting Hemodynamic Parameters and Prevaling Level of Plasma Renin Activity in Unanesthetized Rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>PRA ng/hr/ml</th>
<th>SBP mm Hg</th>
<th>DBP mm Hg</th>
<th>HR beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>8.0 ± 1.0</td>
<td>212 ± 3.4</td>
<td>158 ± 2.3</td>
<td>470 ± 4.9</td>
</tr>
<tr>
<td>WKY</td>
<td>4.8 ± 1.0</td>
<td>152 ± 2.4*</td>
<td>113 ± 2.4*</td>
<td>383 ± 11.7*</td>
</tr>
<tr>
<td>APW</td>
<td>5.7 ± 1.5</td>
<td>148 ± 2.9*</td>
<td>111 ± 2.8*</td>
<td>466 ± 10.8</td>
</tr>
</tbody>
</table>

SBP = systolic arterial pressure; DBP = diastolic arterial pressure; PRA = plasma renin activity; HR = heart rate; SHR = spontaneously hypertensive rat; WKY = Wistar-Kyoto, APW = Alderley Park Wistar rat. Data given are means ± sem; n = 23 to 26.

*p < 0.001; significantly different from SHR by analysis of variance.

*fp < 0.001; significantly different from APW by analysis of variance.
was clearly a cumulative effect over the first 3 days, which was not seen in the APW, in which captopril also lowered blood pressure by 15 to 20 mm Hg over the 5-day period. The percent fall in blood pressure in these two rat strains was approximately 20% in the SHR and 12% in the APW after 3 to 4 days. In WKY no hypotensive response was seen. Captopril had no effect on the heart rate of any of the three strains; the decreases in pressure caused by captopril in SHR and APW were therefore not accompanied by a reflex tachycardia.

Effect of Captopril Administered Intravenously to SHR, WKY, and APW

Captopril (1 mg/kg i.v.) reduced blood pressure in both SHR and APW. At 10 minutes after dosing, the reduction in mean pressure was 8.6 ± 2.2 mm Hg in SHR \((p < 0.01) (n = 11)\) and 12.3 ± 2.3 mm Hg in APW \((p < 0.001) (n = 8)\). In WKY there was no fall in pressure at this time although there was a small reduction in pressure of 4.6 ± 1.0 mm Hg 5 minutes after dosing \((p < 0.01) (n = 11)\). Saline was without effect.

**FIGURE 1.** Effect of captopril (1.0 mg/kg intravenously) on vasoconstrictor responses to spinal stimulation and norepinephrine in pithed rats. Upper graphs: SHR rats ○ before and ⋄ after captopril \((n = 9)\). Lower graphs: WKY rats △ before and ▲ after captopril \((n = 9)\). ** p < 0.01; *** p < 0.001, significantly different from control value.
Discussion

We have previously demonstrated that captopril interferes with sympathetic vasoconstriction in the APW rat by blocking the production of angiotensin II, which normally facilitates vascular adrenergic neurotransmission.\cite{11,12,13} The present study extends these observations to the SHR and WKY strains of rat and suggests that this effect of captopril may be a major mechanism by which the drug lowers blood pressure. Captopril attenuated the pressor responses evoked by electrical stimulation in the pithed SHR to a greater extent than in the WKY rat. This effect of captopril must be due to interference with the renin-angiotensin system since it did not occur in nephrectomized animals with a low PRA. In addition, previous studies using the APW rat have shown that saralasin has an effect similar to that of captopril, whereas bradykinin did not alter the response to adrenergic nerve stimulation.\cite{12} The greater effect of captopril in attenuating nerve-induced vasoconstriction in the SHR may be explained by the SHR having significantly higher levels of angiotensin I and II than the WKY, or the hypertensive animal being...
more sensitive to the facilitatory effect of angiotensin II on adrenergic vasoconstrictor mechanisms.

The mature SHR has been reported to have high,\textsuperscript{20,21} normal,\textsuperscript{22} or low\textsuperscript{23} levels of PRA. The confusion over this issue could be the result of different ages of animals being used or of the methods used to obtain blood samples. In the present study, blood was taken from conscious unstressed rats, and the results demonstrate that, while the SHR had a higher level of PRA than the WKY, this did not reach statistical significance. It is unlikely, therefore, that this could make a major contribution to the greater effect of captopril in the conscious animals. However, pithing caused a sustained increase in PRA in the SHR. This would be one reason why the effects of captopril are greater in this hypertensive strain.

The experiments using perfused mesenteric arteries demonstrate that preparations from the SHR are more sensitive than those from the WKY rat to the adrenergic potentiating effects of exogenous angiotensin II. Since mesenteric artery preparations from the SHR (in the absence of angiotensin II) responded to nerve stim-
Angiotensin II can potentiate the vasoconstrictor responses evoked by adrenergic nerve stimulation by actions at both pre- and post-junctional sites. The results of this study suggest that increased sensitivity to the potentiating effect of angiotensin II probably occurs at the adrenergic nerve ending. Thus, captopril attenuated the response to nerve stimulation to a greater extent in the pithed SHR than in the WKY rat, whereas responses to exogenous norepinephrine were decreased to the same extent. In addition, previous studies have shown that low concentrations of the octapeptide in vitro potentiate responses to adrenergic nerve stimulation more than those to exogenous norepinephrine. After blockade of the renin angiotensin system with captopril or after nephrectomy, SHR still had a greater pressor response to nerve stimulation than WKY rats; similarly, SHR had a greater mesenteric constrictor response to nerve stimulation in the absence of angiotensin II than WKY. These results thus indicate that SHR might release more catecholamines upon sympathetic nerve stimulation than WKY. Since the WKY responded less to nerve stimulation per se, the attenuation by captopril on a percentage basis was the same in both strains of rat. Nevertheless, this still indicates that captopril has a greater attenuating effect in SHR since SHR has been reported to have greater sympathetic nerve activity than normotensive control rats.

Antonaccio and Kerwin have also recently reported similar results to these in SHR although they could find no inhibition of responses to norepinephrine following an intravenous dose of 10 mg/kg of captopril. After chronic dosing they reported that responses to both nerve stimulation and norepinephrine were inhibited by captopril. They also found no inhibiting effect of saralasin or teprotide, whereas we have previously
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Figure 6. Effect of captopril orally 30 mg/kg for 5 days on blood pressure in unanesthetized rats. A. SHR; ○ = NaCl 0.9%; ● = captopril. B. APW rat; □ = NaCl 0.9%; ● = captopril. C. WKY rat; △ = NaCl 0.9%; ▲ = captopril. Data given are means ± SEM. For captopril treatment, n = 14 to 16. For vehicle treatment, n = 8 to 9. Dosing was carried out at time 0 on each day. * p < 0.05, ** p < 0.01, *** p < 0.001. significantly different from control group.

demonstrated in pithed normotensive rats that captopril, saralasin, and teprotide all inhibit responses to both nerve stimulation and norepinephrine injection.12 This discrepancy is difficult to explain, although other workers also report an inhibiting effect of acute i.v. captopril in response to exogenous norepinephrine.28 It is possible that strain differences could explain the discrepancy in the results since it has recently been reported that the facilitatory action of angiotensin in rat mesenteric artery preparation shows marked variations between different strains of rat.29 We have previously reported that captopril does not attenuate responses to cardiac nerve stimulation in APW rats,12 and Antonaccio and Kerwin25 have also reported this in SHR. It thus appears that the facilitating action of All on sympathetic nerve stimulation is either restricted to or more pronounced in vascular tissue.

Captopril given orally for 5 days reduced the blood pressure in the SHR but not WKY rat, confirming previous work by others.4 A reflex tachycardia was not seen in the SHR, as blood pressure fell following captopril treatment; this may be due to interference with homeostatic reflexes due to angiotensin converting enzyme inhibition, as previously reported.8,30 The reason why captopril should lower blood pressure in the SHR but not WKY is not clear. Several factors could be responsible: 1) in our experiments PRA tended to be higher in SHR than WKY; 2) there was a greater prejunctional sensitivity to angiotensin II in SHR than WKY; and 3) others31,32 have found increased postjunctional reactivity to the direct pressor actions of angiotensin in the SHR.

In the APW, captopril also lowers blood pressure, although to a smaller extent, than in the SHR. Thus, the APW is sensitive to the hypotensive action of captopril, yet the resting blood pressure of the APW is the same as that of the WKY. Also, the PRA of the APW is not significantly different from that of the WKY. As the APW rat is normotensive, it is unlikely that increased reactivity to the postjunctional effects of angiotensin II will occur. Previous work has shown, however, that the APW is very sensitive to the prejunctional actions of angiotensin II and captopril. In conscious SHR and APW, intravenous captopril in the same dose that was used in the pithed rat study also produced a small depressor effect. In WKY, intravenous captopril only transiently lowered pressure. Thus, captopril given either orally or intravenously lowers blood pressure in both SHR and APW, and both these strains are sensitive to the prejunctional actions of angiotensin. This provides strong evidence that the hypotensive actions of captopril in both normotensive and hypertensive strains of rat are due to the interference by the drug with the prejunctional actions of angiotensin II.

The enhanced prejunctional action of angiotensin II is clearly not responsible for the hypertension in the SHR, since this property is shared with the normotensive APW. Therefore, additional factors present in the SHR must be responsible for the elevation in blood pressure.
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