Effects of Captopril on Vascular Noradrenergic Transmission in SHR

DOUGLAS C. EIKENBURG, PH.D.

SUMMARY The effects of captopril, 3 and 10 mg/kg, on vascular noradrenergic transmission were examined in vivo in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). These experiments were performed on mesenteric vascular beds perfused in situ. In WKY, 3 mg/kg captopril failed to significantly lower mean arterial blood pressure (MAP) and also failed to have a significant effect on the frequency-response curve to sympathetic nerve stimulation or dose-response curve to norepinephrine (NE) in the mesentery of WKY. In SHR mesentery, 3 mg/kg captopril failed to alter the frequency-response curve or NE dose-response curve, while it significantly lowered MAP. The higher dose of captopril, 10 mg/kg, also failed to lower MAP in WKY mesentery, although it caused some reduction in pressor responses to sympathetic nerve stimulation and NE. In SHR mesentery, 10 mg/kg captopril significantly lowered MAP and reduced pressor responses to both nerve stimulation and NE. It should be noted, however, that captopril lowered responses to nerve stimulation and NE to a similar degree in both SHR and WKY, and there was no indication of a prejunctional action on vascular noradrenergic transmission. In conclusion, although captopril was more effective in lowering MAP in SHR than in WKY, no evidence was found for significantly greater facilitation of vascular sympathetic neurotransmission by endogenous angiotensin II in SHR than in WKY, and most of the actions of captopril on vascular neurotransmission appeared to be postjunctional in nature and unrelated to either the renin-angiotensin system or the kallikrein-kinin system. (Hypertension 6: 660-665, 1984)

KEY WORDS • captopril • norepinephrine release • angiotensin • spontaneously hypertensive rat • Wistar-Kyoto rat

Effects of Captopril on Vascular Noradrenergic Transmission in SHR

DOUGLAS C. EIKENBURG, PH.D.

SUMMARY The effects of captopril, 3 and 10 mg/kg, on vascular noradrenergic transmission were examined in vivo in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). These experiments were performed on mesenteric vascular beds perfused in situ. In WKY, 3 mg/kg captopril failed to significantly lower mean arterial blood pressure (MAP) and also failed to have a significant effect on the frequency-response curve to sympathetic nerve stimulation or dose-response curve to norepinephrine (NE) in the mesentery of WKY. In SHR mesentery, 3 mg/kg captopril failed to alter the frequency-response curve or NE dose-response curve, while it significantly lowered MAP. The higher dose of captopril, 10 mg/kg, also failed to lower MAP in WKY mesentery, although it caused some reduction in pressor responses to sympathetic nerve stimulation and NE. In SHR mesentery, 10 mg/kg captopril significantly lowered MAP and reduced pressor responses to both nerve stimulation and NE. It should be noted, however, that captopril lowered responses to nerve stimulation and NE to a similar degree in both SHR and WKY, and there was no indication of a prejunctional action on vascular noradrenergic transmission. In conclusion, although captopril was more effective in lowering MAP in SHR than in WKY, no evidence was found for significantly greater facilitation of vascular sympathetic neurotransmission by endogenous angiotensin II in SHR than in WKY, and most of the actions of captopril on vascular neurotransmission appeared to be postjunctional in nature and unrelated to either the renin-angiotensin system or the kallikrein-kinin system. (Hypertension 6: 660-665, 1984)

KEY WORDS • captopril • norepinephrine release • angiotensin • spontaneously hypertensive rat • Wistar-Kyoto rat

IT is generally accepted that the angiotensin-converting-enzyme inhibitor captopril is more effective in lowering blood pressure in spontaneously hypertensive rats (SHR) than in normotensive Wistar-Kyoto rats (WKY).1,2 The reason for this difference remains unclear, since most evidence indicates that plasma renin activity (PRA) in the SHR is within the normal range.3-5 However, several reports have suggested that vascular wall renin, and not plasma renin activity, may be responsible for the greater effectiveness of captopril in SHR.6,7 Recently, it has been hypothesized that the greater hypotensive action of captopril in SHR may be related to a greater prejunctional facilitatory action by angiotensin II (ANG II) at the vascular sympathetic nerve terminal.6,8 This being the case, similar amounts of ANG II would cause a greater amount of norepinephrine (NE) to be released at the level of the blood vessel in SHR than in WKY, and removal of this influence of angiotensin by captopril would have a greater effect on blood pressure in SHR than in WKY. Both in vitro and in vivo, exogenous ANG II causes a greater degree of potentiation of pressor responses elicited by sympathetic nerve stimulation in vascular tissue of SHR than in WKY.10,11 Furthermore, captopril decreases pressor responses to spinal stimulation in the pithed SHR to a greater extent than pressor responses to exogenous NE. A similar dose of captopril in the pithed WKY has minimal effects on pressor responses to nerve stimulation.

Although some conflicting results have been reported, investigators have observed that the actions of captopril on nerve stimulation responses are eliminated by prior nephrectomy.12,13 These observations have led to the suggestion that captopril lowers pressor responses to spinal stimulation in the pithed SHR by eliminating the prejunctional facilitatory actions of endogenous ANG II on vascular noradrenergic transmission. In addition, since angiotensin produces greater facilitation in SHR than in WKY, this mechanism has been suggested as the reason why captopril is a more effective hypotensive agent in SHR.1

Existing evidence indicates that endogenous ANG II has a prejunctional action in the pithed SHR, but several questions remain. First, since PRA in the pithed rat is reportedly very high (at least 30 ng of angiotensin I (ANG I) generated per milliliter of plasma per hour),
are the findings in the pithed rat applicable to other experimental situations where captopril has been shown to be a more effective hypotensive agent in SHR than in WKY? Second, pressor responses in the pithed rat are the result of changes in venous compliance and cardiac output as well as increases in arterial resistance. Thus, the level of the vascular tree at which captopril is acting remains uncertain. For these reasons, the effects of captopril on vascular noradrenergic transmission in SHR and WKY have been investigated with another in vivo experimental model, the in situ blood-perfused rat mesentery. This model has the following advantages: 1) PRA is not as high as in the pithed rat; 2) the effects of exogenous ANG II have been characterized in this vascular bed both in vitro and in vivo in SHR and WKY; and 3) the rat mesentery preparation allows the study of the effects of captopril in vivo in a single arterial bed.

**Methods**

We obtained 18- to 22-week-old male SHR and age- and sex-matched Wistar-Kyoto normotensive controls from Taconic Farms Inc. (Germantown, New York). The rats were allowed to acclimate to our facilities for 1 week before the experiments were begun. Prior to the experiments the systolic blood pressure of each animal was determined by the tail-cuff method.

**In Situ Blood-Perfused Rat Mesentery**

The method of Jackson and Campbell was used. Briefly, rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and the trachea and jugular vein were cannulated. An extracorporeal circuit was established between the abdominal aorta and the superior mesenteric artery. Flow in this circuit was maintained at 2 ml/min by a Harvard peristaltic pump. Arterial pressure was monitored from the extracorporeal circuit as was mesenteric perfusion pressure, an index of mesenteric vascular resistance under constant flow conditions. The superior mesenteric artery was cut behind the point of cannulation as were the nerves innervating the mesentery and the mesenteric artery, and its innervation was placed over bipolar platinum electrodes that stimulated the sympathetic nerves to this vascular bed. Control experiments indicated that responses to such stimulation were completely eliminated by guanethidine. An injection port was included in the extracorporeal circuit to permit intraarterial injections of NE into the mesentery.

The protocol for the experiments was as follows. After a 30-minute equilibration period, a frequency-response curve to periartrial nerve stimulation (40 V, 1 msec, 30 sec, 1–40 Hz) and a dose-response curve to NE were generated in the mesentery. Then, captopril, 3 or 10 mg/kg, was administered intravenously. After 10 minutes for stabilization of aortic and mesenteric pressures, a frequency-response curve and NE dose-response curve were again generated. Pilot experiments demonstrated that the doses of captopril used were sufficient to reduce pressor responses to ANG I by more than 90%. Furthermore, saline control experiments demonstrated that responses to nerve stimulation and NE were reproducible over the duration of these experiments.

**Plasma Renin Activity**

The PRA was measured in representative groups of WKY and SHR. Mesenteric perfusion was established as described above, and the appropriate equilibration period was observed. Then, rather than generate a frequency-response curve and a dose-response curve, an arterial blood sample was taken from the extracorporeal circuit for PRA determination. Blood samples were centrifuged at 4°C, and the plasma was frozen until the time of assay. The PRA, measured as nanograms of ANG I generated per milliliter of plasma per hour, was determined at pH 6.0, 37°C, and ANG I was quantified with a Squibb ANG I radiolmmunoassay kit.

**Data Analysis**

Control and captopril-treated frequency-response curves and NE dose-response curves were compared in each group of animals by a two-way analysis of variance (ANOVA), randomized complete block design. Mean control and treated responses to each frequency or NE dose were compared with a Students-Newman-Keuls test. Blood pressures and perfusion pressures were compared both within groups and between groups by ANOVA randomized complete block design. All significant differences in this study are indicated at the level of p < 0.05.

**Results**

As determined by the tail-cuff method, systolic arterial pressures in the SHR and WKY rats before the perfusion experiments were 179 ± 8.7 and 123 ± 7.4 mm Hg, respectively. The mean arterial blood pressure (MAP) and mesenteric perfusion pressure in anesthetized SHR and WKY are shown in Table 1. In both

| Table 1. Effects of Captopril on Mean Blood Pressure and Mesenteric Perfusion Pressure in WKY and SHR |
|------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Rat     | Captopril dose (mg/kg) | Mean arterial pressure (mm Hg) | Mean perfusion pressure (mm Hg) |  |
|         |                  | Control       | Captopril     | Control       | Captopril     |  |
| WKY (n = 5) | 3               | 82.4 ± 3.8    | 65.6 ± 6.3    | 37.4 ± 0.8    | 36.0 ± 0.4    |  |
| SHR (n = 5)  | 3               | 136.6 ± 7.2*  | 102.2 ± 9.9†  | 53.0 ± 1.5*   | 52.3 ± 2.3    |  |
|     | 10              | 80.6 ± 6.2    | 72.1 ± 5.6    | 42.6 ± 3.5    | 41.6 ± 4.3    |  |
|     | 10              | 134.7 ± 5.3*  | 90.3 ± 6.5†   | 58.6 ± 2.8*   | 49.8 ± 2.6†   |  |

*Significantly different from WKY.
†Significantly different from control.
conscious and anesthetized rats, the arterial pressures were significantly greater in SHR than in WKY. Furthermore, mesenteric perfusion pressure was greater in the SHR, which indicated that the baseline vascular resistance in the mesentery, which was not under the influence of higher brain centers in this preparation, was greater in SHR. Also shown in Table 1 are the blood pressures and perfusion pressures in SHR and WKY after captopril. In WKY, neither 3 nor 10 mg/kg captopril significantly lowered MAP. This is in contrast to SHR, in which both doses produced a significant reduction in MAP. With regard to mesenteric perfusion pressure, captopril failed to significantly alter this parameter at either dose in WKY. In SHR, 3 mg/kg did not lower the perfusion pressure, but 10 mg/kg did significantly. However, it should be noted that after captopril, perfusion pressure remained significantly higher in SHR than in WKY.

The effects of 3 mg/kg captopril on the frequency-response curve to nerve stimulation and dose-response curve to NE in WKY are shown in Figure 1. Captopril had no significant effect on pressor responses to nerve stimulation or NE as determined by ANOVA. The effects of 3 mg/kg captopril, in SHR are shown in

![Figure 1](image-url)

**Figure 1.** Effects of captopril, 3 mg/kg i.v., on vasoconstrictor responses to periarterial nerve stimulation and norepinephrine in the in-situ perfused mesentery of WKY rats. Pressor responses are represented as increases in perfusion pressure in the mesentery under constant flow conditions. The symbols and vertical lines represent the mean ± SEM, respectively. No significant differences were observed.

![Figure 2](image-url)

**Figure 2.** Effects of captopril, 3 mg/kg i.v., on vasoconstrictor responses to periarterial nerve stimulation and norepinephrine in the in-situ perfused mesentery of SHR rats. Pressor responses are expressed as increases in perfusion pressure in the mesentery under constant flow conditions. The symbols and vertical lines represent the means ± SEM, respectively. The asterisk indicates a significant difference between the mean responses obtained at the indicated dose or frequency before vs after captopril; p < 0.05.
Figure 2. In SHR, this dose of captopril did not significantly alter pressor responses to either nerve stimulation or NE.

To eliminate the possibility that captopril failed to alter responses to nerve stimulation because of an insufficient dose of captopril, additional experiments were performed with a higher dose of captopril, 10 mg/kg. The results of these experiments in WKY are shown in Figure 3. Captopril significantly reduced pressor responses to both nerve stimulation and NE in WKY. The effect on NE responses appeared to be slightly greater. The results from similar experiments in SHR are shown in Figure 4. In these experiments, captopril decreased responses to both nerve stimulation and NE. It should be pointed out that the magnitudes of the effects on nerve stimulation and NE are similar, which suggests that the decreases in response to nerve stimulation were probably the result of decreased vascular responsiveness to NE.

Although blood pressure was significantly higher in SHR than in WKY and captopril reduced blood pressure to a much greater extent in SHR than in WKY, PRA was not significantly different in SHR and WKY: 17.0 ± 3.4 ng ANG I/ml/hr in WKY and 16.9 ± 1.7 ng ANG I/ml/hr in SHR.

**Figure 3.** Effects of captopril, 10 mg/kg i.v., on vasoconstrictor responses to periarterial nerve stimulation and norepinephrine in the in-situ perfused mesentery of WKY rats. Pressor responses are represented as increases in perfusion pressure in the mesentery under constant flow conditions. The symbols and vertical lines represent the means ± SEM, respectively. The asterisk indicates a significant difference between the mean responses obtained at the dose or frequency indicated before vs after captopril: p < 0.05.

**Figure 4.** Effects of captopril, 10 mg/kg i.v., on vasoconstrictor responses to periarterial nerve stimulation and norepinephrine in the in-situ perfused mesentery of SHR rats. Pressor responses are represented as increases in perfusion pressure in the mesentery under constant flow conditions. The asterisk indicates a significant difference between the mean responses obtained at the dose or frequency indicated before vs after captopril: p < 0.05.
Discussion

The results of the present study confirm the observations of numerous investigators, which indicate that captopril is a much more effective hypotensive agent in SHR than in WKY. In our study, captopril failed to significantly lower blood pressure in WKY, while it reduced blood pressure in SHR by 20% to 30%. It should be pointed out, however, that after captopril the blood pressure remained significantly higher in SHR than in WKY. The reason for the greater effectiveness in SHR remains unclear, since PRA was not significantly higher in SHR than in WKY. Perhaps it is the result of greater vascular reactivity to ANG II in SHR. A portion of the hypotensive action of captopril in SHR can be attributed to a decrease in peripheral resistance independent of neural mechanisms, since at the higher dose captopril lowered perfusion pressure in the denervated mesenteric bed of SHR. At the lower dose of captopril, however, blood pressure was reduced without significant effects on mesenteric resistance. Thus, it would appear that captopril lowers blood pressure in SHR by other mechanisms in addition to eliminating the influence of ANG II on vascular smooth muscle.

Since it had been reported that captopril decreased pressor responses to nerve stimulation in the pithed rat with minimal effects on pressor responses to exogenous NE, an effect on sympathetic vascular transmission of a prejunctional nature had been suggested. Our present study was designed to examine this possibility using the in-situ perfused rat mesentery as an experimental model. In our study, 3 mg/kg captopril, which produced significant MAP reductions in SHR, had no effect on pressor responses to either sympathetic nerve stimulation or exogenous NE. Captopril also failed to affect responses in WKY at this dose. When the dose of captopril was increased to 10 mg/kg, a significant decrease in responses to both nerve stimulation and NE was observed in both WKY and SHR. However, the data indicate that captopril had no preferential effect on vasoconstrictor responses to nerve stimulation, in contrast to what had been reported in the pithed rat. Our results suggest that captopril reduced vasoconstrictor responses to NE in the mesentery and in this way reduced responses to nerve stimulation. No evidence of prejunctional action by ANG II was observed.

The mechanism by which captopril reduced vasoconstrictor responses to NE in this study is not clear. Several groups of investigators have found that ANG II, both in vitro and in vivo, can increase responses to exogenous NE. The mechanisms suggested for this effect include blockade of NE reuptake and/or changes in the reactivity of the vascular smooth muscle to NE by an unknown mechanism. Either of these mechanisms or a combination of both may explain why captopril lowered responses to NE in this study. However, in SHR it is probable that at least part of the reduction in NE responses at the high dose of captopril may be the result of the lower baseline resistance present after captopril, as indicated by the reduced perfusion pressure. This effect on perfusion pressure, we feel, is a nonspecific effect of captopril and not a result of converting-enzyme inhibition, since lower doses of captopril were sufficient to eliminate the pressor response to exogenous ANG I without affecting perfusion pressure. A nonspecific effect of high doses of captopril on both vascular tone and on responsiveness to NE has been suggested by several previous investigations.

With regard to the effects of captopril on vascular sympathetic transmission, our data do not agree with the observations of others in the pithed rat. Several explanations can be offered. First, PRA in the pithed rat, as stated earlier, has been shown to be 30 ng ANG I/ml/hr or greater. In our experiments, PRA was higher than that reported in conscious animals, but at 17 ng ANG I/ml/hr it was significantly lower than that observed in pithed rats. It is possible that the high PRA observed in pithed animals is necessary to demonstrate a prejunctional facilitatory action by endogenous ANG II. Second, pressor responses to spinal stimulation in the pithed rat are not due solely to increases in peripheral arterial resistance. It has been shown recently that pressor responses to NE in the pithed rat are the result of decreases in venous capacitance and increases in cardiac output, as well. Thus, the effect of captopril observed in pithed rats may be due to actions at sites other than arterial neuroeffector junctions. We do not feel that a reasonable alternative to explain our data would be a lack of responsiveness to endogenous ANG II in the mesenteric vascular bed. This bed has been more extensively studied than perhaps any other in the rat with regard to the actions of ANG II. The ability of ANG II to facilitate responses to nerve stimulation at concentrations that have little or no effect on responses to NE has been demonstrated repeatedly both in vitro and in vivo. It should be added that concentrations of ANG II that potentiate vasoconstrictor responses to nerve stimulation in vivo in the rat mesentery also produce a slight elevation of systemic pressure. Thus, if captopril acts to lower blood pressure in SHR by eliminating the prejunctional facilitatory action of endogenous ANG II, we should have observed this effect in our experiments. One might also argue that, since NE release was not measured in this study, a prejunctional facilitatory action by ANG II cannot be ruled out. This is certainly true. However, our data indicate that if angiotensin has such an action in SHR, the influence of this action on the ability of the sympathetic nerves to cause vasoconstriction is minimal.

In conclusion, the data presented here suggest that endogenous ANG II has little if any effect on vascular transmission in the in-situ perfused mesentery of SHR, as indicated by the effects of captopril. Since this bed has been shown by several investigators to be sensitive to the prejunctional effects of ANG II at subpressor doses, our data suggest that mechanisms other than a prejunctional action on arterial sympathetic neurotransmission may be responsible for the greater hypotensive action of captopril in SHR.
Acknowledgments
The author would like to express appreciation to Philomena Kong for her excellent technical assistance and to Belinda Suarez of the Word Processing Center for the transcription of this manuscript.

References
Effects of captopril on vascular noradrenergic transmission in SHR.
D C Eikenburg

Hypertension. 1984;6:660-665
doi: 10.1161/01.HYP.6.5.660

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/6/5/660