Long-term Captopril Treatment
Angiotensin II Receptors and Responses

KAREN M. WILSON, WELLS MAGARGAL, AND KATHLEEN H. BERECEK

SUMMARY The purpose of this study was to elucidate the mechanism of the antihypertensive effect of the angiotensin I (Ang I) converting enzyme inhibitor captopril in spontaneously hypertensive rats (SHR). Drinking responses, peripheral vascular reactivity, and angiotensin II (Ang II) receptor binding in both the brain and vascular smooth muscle were examined in control and captopril-treated SHR. Pregnant and nursing dams were treated with oral captopril (100 mg/kg). After weaning, offspring were maintained on captopril (50 mg/kg). The average systolic pressures after 21 weeks of captopril treatment were 122 ± 3 mm Hg (male) and 118 ± 4 mm Hg (female) as compared with 169 ± 4 mm Hg (male) and 162 ± 2 mm Hg (female) in age-matched controls. Drinking responses to intracerebroventricular (10 ng) and subcutaneous (100 μg/kg) administration of Ang I and II were attenuated in captopril-treated SHR in comparison to control SHR. Ang II receptor binding in the hypothalamus, thalamus, and septum of captopril-treated SHR was also significantly reduced. In contrast to a depressed angiotensinergic system in the brain, peripheral vascular reactivity to Ang II, as determined in isolated, artificially perfused kidneys, was elevated. Threshold and ED₅₀ values for Ang II were significantly lower in captopril-treated SHR than in controls. Ang II receptor binding in aortic smooth muscle cells prepared from captopril-treated SHR was also significantly greater than in cells from controls. Thus, lifetime treatment with captopril induced alterations in the renin-angiotensin systems in the periphery and brain that were manifested by changes in receptor binding and responsiveness to Ang II. However, the effects of treatment were not the same on vascular and central Ang II receptors, suggesting differential regulation.

(Hypertension 11 [Suppl I]: I-148-I-152, 1988)

KEY WORDS • renal vascular reactivity • aortic smooth muscle cells • drinking response • angiotensin II receptors • spontaneously hypertensive rats

ANATOMICAL and functional evidence suggest that the brain renin-angiotensin system (RAS) may play a role in the pathogenesis of hypertension. In comparison to their controls, spontaneously hypertensive rats (SHR) show increases in renin activity¹ and angiotensin II (Ang II)–like immunoreactivity in the cerebrospinal fluid (CSF).² Vasopressin and adrenocorticotropic hormone (ACTH) secretion and sympathetic tone are also enhanced.³⁴ Additional reports indicate that the pressor effects of centrally administered Ang II are increased in SHR,⁵ and the sensitivity to iontophoretically applied Ang II is enhanced,⁷ and increases in the number of Ang II receptors are observed in areas of the brain that are integral to cardiovascular control.⁸ The strongest evidence that brain Ang II may play a role in the pathogenesis of hypertension is that both short-term or long-term intracerebroventricular (i.c.v.) administration of either the specific Ang II receptor antagonist saralasin¹⁰ or the angiotensin I (Ang I) converting enzyme inhibitor captopril¹³ lowered blood pressure in SHR at doses that were not effective when administered intravenously. These studies suggest an activated RAS in the brain of the SHR model, but the mechanisms underlying these alterations are unclear. Thus, the present studies were designed to determine the role of the brain RAS in the antihypertensive effect of captopril.

Specifically, experiments were conducted to establish whether long-term administration of captopril could modulate central Ang II receptors and subsequently effect central responsiveness to Ang II. Alternatively, these studies examined the ability of alterations in the concentration of Ang II in the brain to influence its receptors. In addition, peripheral vascular responsiveness to Ang II, vasopressin, and norepinephrine was analyzed to determine the effect of long-term
CAPTOPRIL AND ANGIOTENSIN II RECEPTORS/Wilson et al.

Captopril treatment on peripheral mechanisms involved in hypertension and its relationship to alterations of angiotensinergic mechanisms in the brain.

Materials and Methods

Experimental Protocol

Eight-week-old male and female SHR, which were purchased from a virus-free colony (Charles River Breeding Laboratories, Wilmington, MA, USA) were used as breeders for these studies. At the time of breeding, female SHR were given captopril (Squibb Institute, Princeton, NJ, USA) in their drinking water at a dosage of 100 mg/kg/day. They were maintained on this dosage throughout pregnancy and lactation.

After weaning, the SHR pups were maintained on 50 mg/kg/day of captopril in their drinking water for 21 weeks.

Beginning at 7 weeks of age, systolic blood pressure was monitored twice weekly by indirect tail-cuff method in conscious, restrained rats prewarmed at 37°C for 5 to 10 minutes. In half of the rats (n = 10: 5 control, 5 captopril-treated), drinking responsiveness and vascular reactivity were tested between the 19th and 21st weeks of age. The other half (n = 10) was analyzed for Ang II receptor binding in the brain at 21 weeks. The drinking tests and receptor binding analyses were performed only in male rats to avoid any controversies concerning the effect of estrogen on water intake and Ang II receptor binding.

Measurement of Drinking Response

Each rat was given a subcutaneous (s.c.) injection of either Ang I or Ang II (100 μg/kg; Sigma Chemical, St. Louis, MO, USA), and placed in an individual stainless steel metabolism cage. No food was available during the experiment. Water intakes were measured at 30, 60, and 120 minutes after administration of the peptide. Three days were allowed between drinking tests.

The rats then received i.c.v. cannulas (lateral ventricle) to test the drinking response to central administration of either Ang I or II (10 ng i.c.v.). For this purpose, an injector cannula was attached to a 10-μl Hamilton syringe by PE-10 tubing, and filled with Ang II dissolved in sterile isotonic saline. Each rat was injected with 10 ng of Ang II in a volume of 2 μl. Water intake was measured as described above.

Vascular Reactivity

To test peripheral Ang II responsiveness, vascular reactivity to Ang II, vasopressin (Bachem Laboratories, Torrence, CA, USA) and norepinephrine (Winthrop-Breon Laboratories, New York, NY, USA) was measured in isolated, perfused kidneys as previously described. The tissue was perfused with modified Krebs-Henseleit solution containing Ficol 70 U (35 g/L, Sigma Chemical) at a constant flow rate of approximately 5 ml/g kidney weight/min in a single-pass system. The perfusion medium was aerated with a mixture of 95% O₂, and 5% CO₂, and maintained at a pH of 7.4 at 37°C. Perfusion pressure was measured from the side arm of the arterial perfusion cannula (Century P23 DB transducer, Century Technological Co., Englewood, CA, USA) and continuously recorded on a Hewlett-Packard 7758B system polygraph (Hewlett-Packard, Palo Alto, CA, USA). Cumulative dose-response curves were generated for each of the drugs, which were tested in order (norepinephrine, vasopressin, and Ang II) in each kidney.

Receptor Binding Studies in the Hypothalamus, Thalamus, and Septum

The dissection of the brain of the rat and the receptor binding assay were essentially the same as those described by Sirett et al. and Wilson et al. The block of tissue (mean weight, 100 mg) included the hypothalamus, thalamus, and septum (HTS); dipeptidase inhibitors, 0.1 mM phenylmethylsulfonyl fluoride and 100 kallekrein units of aprotinin (both from Sigma Chemical), were added to protect against degradation. The results are expressed as specific binding, which was obtained by subtracting nonspecific binding from total counts. The specific binding of 125I-Ang II for the hypothalamic block of tissue used in these experiments was 75 to 80% of the total counts bound. Protein concentration of the brain particulate fraction was determined by the Lowry method, and results were expressed as femtomoles per milligram of protein. The 125I-Ang II was prepared in our laboratory by the method of Dusterdieck and McElwee, with resulting specific activities between 1500 and 1800 μCi/μg.

Receptor Binding in Aortic Smooth Muscle Cells

Ang II receptor binding was measured in smooth muscle cells prepared from the aorta of control and captopril-treated SHR. The aortic cell cultures were prepared as described previously. Approximately 10⁶ cells were obtained per aorta, and confluency was reached within 13 days. Analysis of Ang II receptor binding was performed at this time. Cells were gently dissociated with collagenase ( Worthington Biochemicals, Freehold, NJ, USA) and elastase ( Sigma Chemical), centrifuged, and resuspended in phosphate-buffered saline (PBS) at a concentration of 4 x 10⁶ cells/ml. For the Ang II receptor binding assay, 250 μl (1 x 10⁶ cells) of the aortic cell suspension were combined with 250 μl of the reaction mixture containing PBS, 1.0 nM 125I-Ang II, bovine serum albumin (Sigma Chemical), and the dipeptidase inhibitors. The reaction proceeded for 45 minutes, and bound and free Ang II was separated by filtration.

Statistical Analysis

Results are expressed as means ± standard error. For drinking and receptor binding results, statistical significance was calculated by Student's t test. The dose-response curves for the reactivity studies were evaluated by analysis of variance. The threshold (ED₀) and ED₉₀ values for each individual dose-response curve were determined through probit analysis and averaged for each group of rats.
TABLE 2. Effect of Lifetime Treatment of Captopril on the Specific Binding of \(^{125}\text{I}-\text{Ang II}\) in the Hypothalamus, Thalamus, and Septum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(^{125}\text{I}-\text{Ang II}) (n = 5)</th>
<th>(^{125}\text{I}-\text{Ang II}) (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.9 ± 0.1</td>
<td>16.4 ± 0.4</td>
</tr>
<tr>
<td>Captopril</td>
<td>2.8 ± 0.1*</td>
<td>13.8 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE (expressed as fmol/mg protein).

*p < 0.01, †p < 0.05, compared with control.

Results

After 21 weeks of treatment with oral captopril, the average systolic blood pressure of both male (122 ± 3 mm Hg; n = 5) and female (118 ± 4 mm Hg; n = 10) SHR was significantly lower (p < 0.01) in comparison to their age-matched controls (169 ± 4 and 162 ± 2 mm Hg for males and females, respectively; n = 10/group) and within the normotensive range of blood pressure. No significant differences were observed in basal heart rates, although they tended to be lower in the captopril-treated group. The heart rates (beats/min) were 486 ± 11 (male) and 492 ± 12 (female) in controls, and 426 ± 17 (male) and 447 ± 14 (female) in captopril-treated SHR. Captopril-treated SHR were also significantly smaller than control SHR (p < 0.01). Captopril-treated males weighed 272 ± 7 g and females weighed 179 ± 2 g. Control weights were 346 ± 6 g (male) and 201 ± 3 g (female; p < 0.01 for both).

Lifetime treatment with captopril did not alter basal water intake between treated (122 ± 7 ml/kg) and control SHR (120 ± 10 ml/kg), but the dipsogenic responsiveness to both i.c.v. (10 ng) and s.c. (100 \(\mu\)g/kg) administration of Ang I and II was significantly attenuated by treatment with captopril (Table 1). Similarly, the binding of Ang II to its receptors in the HTS was also significantly reduced at 0.18 and 0.76 nM \(^{125}\text{I}-\text{Ang II}\) in captopril-treated SHR (Table 2).

In contrast to the alterations observed in the central nervous system, renal vascular responsiveness to Ang II was enhanced as a consequence of long-term captopril treatment. As observed in Figure 1A, the renal vascular dose-response curve to Ang II was shifted to the left in captopril-treated SHR in comparison to control SHR. In addition, the threshold value for Ang II (as signified by ED\(_{0.5}\)) and the ED\(_{0.5}\) value were significantly lower in captopril-treated rats. However, this enhanced vascular response appeared to be specific for Ang II. Renal vascular reactivity to norepinephrine (Figure 1B) and vasopressin (Figure 1C) was reduced as a result of long-term treatment with captopril. Vascular response curves for both norepinephrine and vasopressin were shifted to the right, and threshold and ED\(_{0.5}\) values were significantly greater in captopril-treated SHR than in control SHR.

The alterations in Ang II receptor binding in aortic smooth muscle cells paralleled the enhanced vascular responsiveness to Ang II. At 0.75 nM \(^{125}\text{I}-\text{Ang II}\), significant increases were observed in Ang II receptor binding on aortic cells prepared from captopril-treated SHR (21.9 ± 1.24 fmol/mg protein) in comparison to cells prepared from control SHR (12.5 ± 0.15 fmol/mg protein; p < 0.01).

Discussion

Lifetime oral treatment with captopril significantly attenuated the development of hypertension in SHR. Decreases in Ang II receptor binding in the hypothalamic region of the brain and dipsogenic responsiveness to Ang II accompanied the reduction in blood pressure. The drinking response to Ang II has been identified as a specific, centrally mediated effect of Ang II. Most studies indicate that this area of the brain (HTS) mediates both the blood pressure and dipsogenic effects elicited by Ang II. The reduced drinking responsiveness to Ang I observed in this study suggests that the conversion of Ang I to Ang II was inhibited by captopril. However, the observations that the drinking responses to Ang II were also depressed suggest that the decreased responsiveness may be related to the reduction of Ang II receptors in the brain. The results are consistent with those of Schelling and Felix, who reported that lifetime captopril treatment decreased Ang II receptor sensitivity to iontophoretically applied Ang II.

In contrast to the central effects, peripheral responsiveness to Ang II and receptor binding kinetics of the peptide were significantly enhanced as a result of lifetime captopril treatment. In comparison to the hypertensive controls, captopril-treated SHR showed increased renal vascular reactivity to Ang II; moreover, Ang II receptor binding was significantly enhanced in the aortic cells of these rats. Thus, a decrease in the circulating levels of Ang II differentially affected peripheral and central receptors.

The effects of alterations in circulating Ang II levels on peripheral vascular receptors have been well established. A decrease in Ang II levels produces an increase...
in vascular Ang II receptors, whereas, an elevation of Ang II levels is accompanied by a decrease in receptor number. The ability of Ang II to regulate receptors in the brain remains controversial, however, as increases, decreases, and no changes of Ang II receptors have been observed in the presence of elevated Ang II concentrations. Our study suggests, as have those of other investigators, that captopril may cross the blood-brain barrier and affect Ang II production in the brain. Measurements of Ang II concentration in the brain could help to determine whether this is a direct effect of Ang II levels in the brain. However, the effect of the absence of Ang II on the release of other hormones, such as aldosterone, must also be considered.

Decreases in peripheral vascular reactivity to vasopressin and norepinephrine were also observed as a result of lifetime captopril treatment. We previously reported that long-term i.c.v. administration of the drug to SHR significantly decreased vascular reactivity. This depression in vascular responsiveness occurred at doses of captopril that have no effect when given peripherally and was not due to the decreases in blood pressure produced by the infusions. These studies suggested that the vascular responses may be due to a central effect of captopril.

In the present study, decreases in vascular reactivity to vasopressin and norepinephrine also may be due to a central effect, that is, a result of decreases in the brain angiotensin receptors and responsiveness. Brain Ang II is known to effect the release of vasopressin and ACTH. Thus, the possibility exists that the alterations in vascular reactivity observed in SHR subjected to lifetime administration of captopril may be due to alterations in plasma levels of corticosterone or vasopressin (or both) and the effect of these hormones on vascular responsiveness. The results of this study suggest that lifetime administration of captopril alters the mechanisms of Ang II in the brain. This may contribute to the antihypertensive action of captopril in SHR.

Acknowledgments

We are grateful to Dr. Zola P. Horovitz, the Squibb Institute for Medical Research, Princeton, NJ, for generously supplying the captopril; to Deborah Clark for typing the manuscript; and to Joel Robertson and Gina Coshatt for technical assistance.
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Long-term captopril treatment. Angiotensin II receptors and responses.
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Hypertension. 1988;11:I148
doi: 10.1161/01.HYP.11.2_Pt_2.I148

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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