Effect of In Vitro Administration of Captopril on Vascular Reactivity of Rat Aorta

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SUMMARY The effect of acute administration of captopril, an angiotensin converting enzyme inhibitor, on vascular responses of rings of rat aortic smooth muscle was tested in vitro. Dose-response curves for various vasoactive agents were obtained before and after exposure to captopril (2 x 10^{-6} M) for 30 minutes. In the presence of captopril, contractile responses to angiotensin I (5 x 10^{-10} to 5 x 10^{-6} M) were attenuated significantly, probably as a result of decreased local conversion of angiotensin I to angiotensin II. Contractile responses to angiotensin II (10^{-11} to 5 x 10^{-6} M) were not affected by captopril. All responses to norepinephrine (10^{-4} to 10^{-2} M) and phenylephrine (10^{-4} to 10^{-2} M) were attenuated significantly from control in the presence of captopril. In the presence of the α-adrenergic antagonist, phentolamine, captopril did not affect either the contractile responses to KCl (30 to 100 mM) or the isoproterenol-induced (10^{-6} to 10^{-8} M) relaxation of KCl-depolarized tissue. These results suggest that captopril decreased vascular responsiveness to α-adrenergic agonists but not to β-adrenergic agonists. Low concentrations of bradykinin (10^{-10} to 10^{-8} M) induced contraction in KCl-depolarized tissue while higher concentrations (10^{-7} and 10^{-6} M) induced relaxation. In the presence of captopril, relaxation occurred at all concentrations of bradykinin (10^{-10} to 10^{-4} M), probably as a result of decreased degradation of the bradykinin. These data suggest depression of α-adrenergic responsiveness in vascular smooth muscle as another potential antihypertensive action of captopril.

KEY WORDS • captopril • vascular reactivity • α-adrenergic responsiveness

Captopril is an orally effective antihypertensive agent in humans and experimental animals. Administration of this compound is accompanied by an inhibition of angiotensin converting enzyme. As a consequence of this inhibition, both a reduction in plasma angiotensin II (AII) (a potent vasoconstrictor) concentration and an increase in plasma bradykinin (a potent vasodilator) concentration occur. These changes appear unlikely to account completely for the antihypertensive effect of captopril because this compound has been shown to decrease blood pressure in hypertensive patients and in experimental animals with high, low, or normal plasma renin activity, as well as in the normotensive animal. In addition, doses of captopril that lower blood pressure have been reported to have no effect on plasma bradykinin concentration. These findings have led many investigators to question the inhibition of angiotensin converting enzyme as the sole, or even the major, antihypertensive action of captopril.

Another mechanism by which captopril could contribute to the reduction in blood pressure is by way of a direct action on arterial smooth muscle, which results in decreased vascular reactivity. The studies described here were designed to evaluate the effect(s) of acute administration of captopril on vascular reactivity of rat aortic rings to angiotensin I (A1), AII, KCl, norepinephrine, phenylephrine, isoproterenol, and bradykinin. The results of these studies suggest that captopril can exert an effect on vascular smooth muscle by reducing its responsiveness specifically to α-adrenergic agonists. This decrease in vascular α-adrenergic responsiveness may contribute to the antihypertensive action of captopril.

Methods

Tissue Preparation

Male rats (Sprague-Dawley derived) weighing 420 to 540 g were used for this study. For each experiment, two rats were quickly guillotined and the thoracic aorta from each rat was rapidly excised and placed in a modified Krebs solution aerated with 95% O_2; 5% CO_2. The modified Krebs solution contained in millimoles per liter: NaCl, 128; KCl, 4.7; NaHCO_3,
12.5; MgCl₂, 1.2; KH₂PO₄, 1.2; CaCl₂, 2.5; glucose, 11.1 and Na₂EDTA, 0.01. Loosely adherent connective tissue was removed and two 4 mm lengths (rings) were cut from each aorta. These rings were mounted in 10 ml muscle chambers at the optimal resting tension (8 g)² between two stainless steel hooks, one of which was connected to an F-50 microdisplacement myographic transducer with a model DMP-40 Physiograph recorder. The rings were equilibrated for 2 hours at 37°C, with flushing of the chambers every 20 minutes.

Captopril Dose-Response Experiments

After the 2-hour equilibration period, one ring from each rat was exposed to 10⁻⁴ M phenylephrine for 5 minutes while the other rings were exposed to 80 mM KCl for 5 minutes (standard stimulations). The rings were then allowed to relax back to baseline. The standard stimulations were repeated 3 times and the third response was taken as the precontrol measurement. All rings were then exposed to 10⁻⁴ M captopril for 20 minutes and the standard stimulations were repeated in the presence of 10⁻⁷ M captopril. This procedure was continued for graded concentrations of captopril (10⁻⁸, 10⁻⁷, 10⁻⁶, and 10⁻⁵ M). Following the measurements made in the presence of the highest concentration of captopril (10⁻⁴ M), the rings were washed with drug-free Krebs solution. Thirty minutes later the standard stimulations were repeated for a final time and these final responses were taken as post-control measurements. Contractile responses to both KCl and phenylephrine have been found to be reproducible with time throughout an experiment.

Test for Inhibition of Vascular Converting Enzyme

During the last 30 minutes of the 2-hour equilibration period, 2 × 10⁻⁴ M captopril was added to the Krebs solution baking one ring from each rat, while distilled water, in a volume equal to that of the captopril, was added to the Krebs solution bathing the other rings. Exposure to these solutions was continued throughout the remainder of the experiment. After the 30-minute exposure period, the rings were challenged with increasing concentrations of either AI (10⁻¹⁰, 5 × 10⁻¹⁰, 10⁻⁹, 5 × 10⁻⁹, 10⁻⁸, and 5 × 10⁻⁸ M) or AII (10⁻¹¹, 5 × 10⁻¹¹, 10⁻¹⁰, 5 × 10⁻¹⁰, 10⁻⁹, and 5 × 10⁻⁹ M) at 2-minute intervals.

Vascular Reactivity Experiments

After the 2-hour equilibration period, the rings were challenged with increasing concentrations of a given vasoactive agent at 3-minute intervals. After relaxing back to baseline, the rings were then exposed to 2 × 10⁻⁴ M captopril for at least 30 min and rechallenged with the same vasoactive agent in the presence of captopril. The vasoactive agents used were KCl (10, 20, 30, 45, 60, 80, and 100 mM), norepinephrine (10⁻⁷, 10⁻⁸, 10⁻⁹, and 10⁻¹⁰ M), and phenylephrine (10⁻⁷, 10⁻⁸, 10⁻⁹, and 10⁻¹⁰ M). Also, isoproterenol (10⁻⁸, 10⁻⁷, 10⁻⁶, and 10⁻⁵ M) and bradykinin (10⁻⁸, 10⁻⁷, 10⁻⁶, and 10⁻⁵ M) were tested on tissue partially contracted by KCl-depolarization (20 mM KCl for 10 minutes). In some experiments, to test the effect of α-adrenoceptor blockade on captopril-induced changes in vascular responsiveness to certain agents, 10⁻⁴ M phentolamine was added to the Krebs solution during the last 30 minutes of equilibration and maintained in all solutions during the entire experiment. This concentration of phentolamine was used because it had been shown to decrease contractile responses to 10⁻⁴ M phenylephrine by 95.5%.

Drugs

Captopril was generously supplied by Dr. Z. P. Horovitz of the Squibb Institute for Medical Research. Phentolamine-HCl was generously supplied by Mr. Charles Brownley at Ciba-Geigy. L-Norepinephrine-HCl, L-phenylephrine-HCl, and DL-isoproterenol-HCl were purchased from Sigma Chemical Company. Angiotensin I (human) diacetate, AII (human) acetate, and bradykinin triacetate were purchased from U.S. Biochemicals Corporation.

When increasing concentrations of KCl were used, isotonicity was maintained by an equimolar substitution of KCl for NaCl in the Krebs solution. All other drug solutions were made in double distilled water at 100 times the concentration to be used. They were made on the day of the experiment and stored at 4°C until used. Captopril and phentolamine were added to the Krebs solution prior to addition to the muscle chambers while all other drugs were added directly to the baths.

Analysis of Data

At the end of each experiment, all rings were blotted with filter paper and weighed on an analytical balance. The response was calculated as g tension developed/mg wet tissue weight. For most experiments the response was expressed as a percentage of the maximal tension developed prior to treatment with captopril or as a percentage of the response to 20 mM KCl. Data are presented as the mean and standard error.

The paired t test was used to compare responses prior to treatment with captopril to those in the presence of captopril for the standard stimulations and for all concentrations of each vasoactive agent. Student's t test was used to compare responses to either A1 or AII to those in the presence of captopril. Significance was set at the 95% confidence interval.

Results

Dose-Response Relationship Between Vascular Contractile Responses and Concentration of Captopril

Captopril (10⁻⁴ to 10⁻⁵ M) did not affect contractile responses of 6 aortic rings to 80 mM KCl (fig. 1, open circles). On the other hand, contractile responses of 6
The dose-dependent effect of captopril on aortic contractile responses to $10^{-8}$ M phenylephrine and 80 mM KCl. Responses in the presence of captopril and post-control responses were compared to the respective pre-control response. *$p < 0.05$, **$p < 0.01$, $n = 6$ aortic rings.

Effects of Captopril on Vasoactive Agents

1. **Angiotensin I and Angiotensin II**
   - Exposure of six aortic rings to $5 \times 10^{-10}$ to $5 \times 10^{-4}$ M AI was associated with development of tension (fig. 2A, closed circles). Preexposure of six aortic rings to $2 \times 10^{-4}$ M captopril attenuated significantly this angiotensin I-associated development of tension (fig. 2A, open circles). On the other hand, $2 \times 10^{-4}$ M captopril had no effect on the AII ($10^{-11}$ to $5 \times 10^{-6}$ M)-induced contractile responses (fig. 2B).

2. **Various Vasoactive Agents**
   - Contractile responses of 16 aortic rings to 45, 60, 80, and 100 mM KCl were slightly, yet significantly, attenuated in the presence of $2 \times 10^{-4}$ M captopril (fig. 3A). In 12 aortic rings, this captopril-induced attenuation of contractile responses to the higher concentrations of KCl was abolished when the study was repeated in the presence of $10^{-8}$ M phentolamine (fig. 3B).
Contractile responses of 16 aortic rings to norepinephrine (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M) were significantly attenuated in the presence of 2 × 10^{-4} M captopril (fig. 4). The slope (29.04) of the straight portion of the dose-response curve for norepinephrine (10^{-9} to 10^{-6} M), determined using linear regression analysis, was found to be significantly (p < 0.001) less in the presence of 2 × 10^{-4} M captopril (23.08). In the presence of 2 × 10^{-4} M captopril, contractile responses of 16 aortic rings to all concentrations (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) of phenylephrine were attenuated significantly (fig. 5). The slope (31.12) of the straight portion of the dose-response curve for phenylephrine (10^{-6} to 10^{-5} M), determined using linear regression analysis, was found to be significantly (p < 0.001) less in the presence of captopril (22.04). Also, in the presence of 2 × 10^{-4} M captopril, isoproterenol (10^{-8}, 10^{-7}, and 10^{-6} M) induced a significant relaxation of 11 KCl-depolarized aortic rings, while higher concentrations (10^{-6} and 10^{-5} M) induced contractile responses that were attenuated compared to untreated controls (fig. 6 A). In the presence of 10^{-5} M phentolamine, isoproterenol (10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} M) induced a relaxation in 12 KCl-depolarized aortic rings (fig. 6 B) which was not affected by 2 × 10^{-4} M captopril (except at 10^{-4} M isoproterenol).

In 12 KCl-depolarized aortic rings, low concentrations of bradykinin (10^{-10}, 10^{-9}, and 10^{-8} M) induced contractile responses while higher concentrations (10^{-7} and 10^{-6} M) induced relaxations (fig. 7, closed circles). However, in the presence of captopril, bradykinin induced only a relaxation of the KCl-depolarized tissue (fig. 7, open circles).
Discussion

Vascular responses of rat aortic rings to various vasoactive agents were studied both before and after the addition of $2 \times 10^{-4}$ M captopril to the tissue bath. This concentration was calculated to approximate the plasma concentration of captopril when 500 mg were given to a 60 kg human, assuming that it freely distributes throughout the extracellular fluid. Although this represents the maximal plasma concentration that would be achieved, it should be noted that, in this study, lower concentrations of captopril ($10^{-7}$ and $10^{-8}$ M) also induced similar decreases in contractile responses to $10^{-6}$ M phenylephrine (fig. 1). Contractile responses to angiotensin I (fig. 2 A, closed circles) are probably the result of conversion of angiotensin I to angiotensin II by angiotensin converting enzyme present in the endothelial cells of the vascular wall. Captopril had no effect on contractile responses to angiotensin II (Figure 2 B); therefore the shift to the right of the angiotensin I dose-response curve in the presence of captopril (fig. 2 A, open circles) was probably a result of decreased formation of angiotensin II rather than a direct effect of captopril on the contractile response to angiotensin II.

Attenuation of angiotensin I, but not angiotensin II-induced contractile responses by captopril indicates that captopril, at a concentration of $2 \times 10^{-4}$ M, is able to inhibit angiotensin converting enzyme in rat aortic smooth muscle. The effect of captopril on vascular responses to bradykinin was tested because bradykinin is inactivated by kininase II, the angiotensin converting enzyme. In the presence of captopril, relaxation, previously seen at only the higher...
concentrations of bradykinin, occurred at all concentrations (fig. 7). This relaxation was probably a result of higher bradykinin concentrations in the presence of captopril due to a decreased rate of inactivation of bradykinin. The possibility that captopril might affect vascular responses to other vasoactive agents was considered next. In these experiments the effect of captopril on vascular smooth muscle, on the contractile mechanism, and on vascular \( \alpha \)- and \( \beta \)-adrenergic responsiveness was studied.

Captopril induced a slight, yet significant, attenuation of the contractile responses to the 4 highest concentrations of KCl tested (fig. 3 A). This attenuation at 80 mM KCl, not seen in figure 1, may be due either to a gradual depolarization of the rings by the graded increase in concentration of KCl or to the longer exposure time to elevated KCl concentrations. This small decrease could be the result of an effect of captopril on the contractile machinery of the smooth muscle cells, the cell membrane potential or permeability, or an \( \alpha \)-adrenergic component of the KCl-induced contractile response. The latter of these three possibilities was tested by repeating the KCl dose-response curves in the presence of phentolamine, an \( \alpha \)-adrenergic blocking agent. Figure 3 B shows that phentolamine abolished the captopril-induced attenuation of contractile responses to KCl. These results suggest that captopril attenuates an \( \alpha \)-adrenergic component of the KCl-induced contraction which may be associated with either stimulation of a small number of adrenergic nerve endings in the vessel wall or a depolarization-dependent activation of \( \alpha \)-adrenergic receptors.

Data presented in figures 1 and 3 indicate that captopril has an effect on \( \alpha \)-adrenergic responses in aortic smooth muscle; therefore, the effect of captopril on adrenergic responsiveness was tested further. Contractile responses to all concentrations of norepinephrine used were significantly attenuated in the presence of captopril (fig. 4). Because norepinephrine has both \( \alpha \)-adrenergic and \( \beta \)-adrenergic properties, this attenuation could have resulted either from a decrease in \( \alpha \)-adrenergic responsiveness or from an increase in \( \beta \)-adrenergic responsiveness in the rat aorta induced by captopril. To test these two possibilities, the effect of captopril on both phenylephrine-induced contractions and isoproterenol-induced relaxations was studied. Captopril attenuated significantly the contractile responses to phenylephrine, a specific \( \alpha \)-adrenergic agonist (fig. 5), further suggesting that it depresses \( \alpha \)-adrenergic responsiveness in vascular smooth muscle.

Although isoproterenol is considered by many to be a specific \( \beta \)-adrenergic agonist, it also appears to have \( \alpha \)-adrenergic properties, especially at higher concentrations, which lead to vascular contractions under the conditions shown in figure 6 A (closed circles). Isoproterenol-induced relaxation in the presence of captopril (fig. 6 A, open circles) could be due either to a decreased \( \alpha \)-adrenergic response unmasking the \( \beta \)-adrenergic response and/or to an increased \( \beta \)-adrenergic responsiveness. In order to test these possibilities the dose-response curves to isoproterenol were repeated in the presence of phentolamine. Figure 6 B shows that the isoproterenol-induced relaxation obtained with phentolamine in the bath does not appear to be affected by addition of captopril. Therefore, captopril does not appear to affect significantly \( \beta \)-adrenergic responsiveness in rat aortic rings. If captopril has an effect on vascular \( \beta \)-adrenergic responsiveness, it would appear to be a very slight, but not significant, attenuation. The lack of isoproterenol-induced relaxation of rings prior to treatment with captopril is probably due to a decrease either in the number or in the activity of the \( \beta \)-adrenoceptors in the aortic smooth muscle from these older rats.

The captopril-induced attenuation of contractile responses to norepinephrine (fig. 4), phenylephrine (fig. 5), and isoproterenol (fig. 6 A) indicated that it significantly decreases \( \alpha \)-adrenergic responsiveness of rat aortic rings in vitro. Preliminary data indicate that contractile responses of rat aortic rings to serotonin are not affected by \( 2 \times 10^{-4} \) M captopril. Thus, attenuation of contractile responses by captopril appears to be fairly specific for \( \alpha \)-adrenergic agonists. The slopes of the straight portions of the dose-response curves and the maximal contractile responses to both norepinephrine and phenylephrine were significantly less in the presence of captopril. If captopril is acting as an \( \alpha \)-adrenoceptor antagonist, then these data indicate that the antagonism is non-competitive. However, we
are not able to determine, from our data, the mechanism by which captopril affects contractile responses to α-adrenergic agonists. Captopril may attenuate these responses via a mechanism independent of a direct action at the α-adrenoceptor. One interpretation of our data could be that the decreased α-adrenergic responsiveness is due, at least partially, to a depressed local formation of angiotensin II. Okuno et al. recently reported a decreased responsiveness of the rat mesenteric vascular bed to norepinephrine in the presence of captopril. In a second experiment, either angiotensin II or bradykinin was added along with the norepinephrine, prior to addition of captopril. In the presence of either angiotensin II or bradykinin, captopril induced a similar decrease in the norepinephrine response. From these findings Okuno et al. suggested that captopril may decrease α-adrenergic responsiveness through a mechanism independent of its ability to inhibit angiotensin converting enzyme.

Another mechanism by which captopril could possibly attenuate vascular contractile responses to α-adrenergic agonists is through an inhibition of either the local synthesis or the physiological effect of certain prostaglandins. Kondo et al. found, in the rat, that PGE₂ potentiated the responses to norepinephrine in certain vascular beds and that indomethacin attenuated responses to norepinephrine in all vascular beds studied. Pfaffman and Chu-Sun found that PGE₁ and PGF₁α potentiated phenylephrine-induce aortic contractile responses. These studies indicate that certain endogenous prostaglandins may act to potentiate the contractile response to α-adrenergic agonists. If captopril antagonized the prostaglandin-induced potentiation of the contractile responses to α-adrenergic agonists, the net result would be an attenuation of these contractions. This possibility, which is only one of many, remains speculative; thus, more detailed studies will be required to ascertain the mechanism by which captopril decreases vascular responsiveness to α-adrenergic agonists.

In summary, these studies show that captopril (2 × 10⁻⁸ M) attenuated angiotensin I-induced contractile responses and augmented bradykinin-induced relaxations in rat aortic rings. These changes were probably due to a decrease in angiotensin converting enzyme activity. In the same tissue, captopril decreased both norepinephrine- and phenylephrine-induced contractile responses without affecting isoproterenol-induced relaxations. These data suggest that captopril attenuates vascular α-adrenergic responsiveness but does not affect β-adrenergic responsiveness. This attenuation may constitute another potential antihypertensive action of captopril.

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