Captopril attenuates adrenergic vasoconstriction in rat mesenteric arteries by angiotensin-dependent and -independent mechanisms

M. G. COLLIS and J. R. KEDDIE

Bioscience II Department, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K.

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Summary

1. Angiotensin-converting enzyme inhibitors can attenuate reflex sympathetic vasoconstriction in vivo. We have investigated the effects of captopril (SQ 14 225) on adrenergic vasoconstrictor mechanisms in isolated, Krebs–Ringer solution perfused, rat mesenteric arteries.

2. Low concentrations of captopril (2 × 10⁻⁶ mol/l) did not alter the vasoconstrictor response evoked by sympathetic nerve stimulation.

3. Exogenous angiotensin I and I₁ did not have a direct vasoconstrictor effect, but caused dose-related increases in the amplitude of responses induced by nerve stimulation.

4. The potentiating effect of angiotensin I was antagonized by captopril (6–7 × 10⁻⁸–2 × 10⁻⁶ mol/l) and by saralasin (10⁻⁸ mol/l). The potentiating effect of angiotensin I₁ was antagonized by saralasin only.

5. In the absence of exogenous peptides high concentrations of captopril (1 × 10⁻⁴–3 × 10⁻⁴ mol/l) antagonized vasoconstrictor responses evoked by sympathetic nerve stimulation and exogenous noradrenaline, but not those evoked by potassium chloride.

6. These results indicate that captopril can have two types of inhibitory effect at the adrenergic neuro-effector junction. High concentrations antagonize responses to noradrenaline and nerve stimulation. This effect is independent of peptide hormones and is unlikely to occur in vivo. Lower concentrations block the local vascular conversion of angiotensin I into II.

As angiotensin II is an important peripheral amplifier of adrenergic vasoconstriction, this effect will also reduce sympathetic vasoconstrictor tone. This latter interaction could explain the inhibitory effect of converting enzyme inhibitors on sympathetic reflexes.

Key words: adrenergic nerve, angiotensin, bradykinin, captopril, converting enzyme inhibitor, noradrenaline, vasoconstriction.

Introduction

Inhibitors of angiotensin-converting enzyme such as captopril (SQ 14 225), have an antihypertensive effect in man and in rats with renal and spontaneous hypertension [1, 2]. This effect is thought to be a consequence of a reduction in the levels of the pressor hormone angiotensin II and of increased levels of vasodilator kinins. It has been demonstrated that converting enzyme inhibitors attenuate reflex sympathetic vasoconstriction [3]. This could be due to either a central or a peripheral effect of the inhibitor. As captopril also decreases the pressor response evoked by electrical stimulation of the spinal efferent nerves in the pithed rat [4], the drug must interfere with peripheral adrenergic neuro-effector mechanisms.

Angiotensin II is known to potentiate vasoconstrictor responses evoked by exogenous and endogenous noradrenaline [5, 6]. In addition, bradykinin can modulate sympathetic vasoconstriction [7, 8]. Therefore, captopril could indirectly depress sympathetic vasoconstriction by altering the levels of these peptides. Alternatively, the drug could have a direct effect at the adrenergic neuro-effector junction, independent
of peptide hormones. We have investigated these possibilities with an isolated perfused mesenteric artery preparation.

Materials and methods

Methods

Male Wistar rats (220–300 g, Alderley Park strain) were anaesthetized with 60 mg of pentobarbitone sodium/kg intraperitoneally (i.p.). The abdomen was opened and the superior mesenteric artery cannulated [9, 10]. The mesenteric artery and arterioles were removed and placed in a bath of Krebs–Ringer solution (37°C). The mesenteric artery preparation was perfused at a flow rate of 6.5 ml/min with a roller pump (Gilson, Minipuls 2). The composition of the Krebs–Ringer perfusate (mmol/l) was NaCl 118.2, KCl 4.7, MgSO, 1.2, KH,PO, 1.2, CaCl2 2.5, NaHCO3 25, calcium disodium ethylenediaminetetra-acetic acid 0.026 and glucose 5.5, aerated with CO2 + O2 (1:19, v/v) and maintained at 37°C. The pH of the perfusate was 7.35; this was not altered by the highest concentration of captopril (3 \times 10^{-8} \text{ mol/l}) used in the experiments. Mesenteric artery preparations were allowed to equilibrate for 1 h before commencing the experiment. The basal perfusion pressure of the preparation stabilized in 5–10 min and remained stable for at least 6 h. Agonist drugs (noradrenaline and KCl) were injected in volumes of 0.02 ml, at 2–5 min intervals, through a rubber tube proximal to the cannula. When KCl was used, the tissue was perfused with Krebs–Ringer solution containing 5 \times 10^{-4} \text{ mol of phentolamine/l} to block the effects of catecholamines released by depolarization of the sympathetic nerves [11]. When the tissue was exposed to angiotensin I or II, bradykinin, saralasin or captopril, they were added to the Krebs–Ringer solution and perfused continuously. The nerves supplying the mesenteric arteries were electrically stimulated (2 ms pulse width, supramaximal voltage 28–32 V, 20 s trains) via peri-arterial platinum electrodes. Electrical stimulation by this method has been shown previously to activate postganglionic adrenergic sympathetic nerves in this preparation [9]. Vasoconstrictor responses were recorded as increases in perfusion pressure, with a pressure transducer and chart recorder.

Stability of the preparation

Responses evoked by submaximal frequencies of electrical stimulation could be repeated without any significant deterioration in their amplitude. It has been shown previously that dose–response curves to noradrenaline are stable and repeatable in this preparation [10]. In all experiments, in which dose–response curves to angiotensin were repeated, the tissues were initially exposed to a high concentration of angiotensin I or II (7.8–9.7 \times 10^{-8} \text{ mol/l}) to stabilize their sensitivity to the peptide.

Experimental protocol

Effect of angiotensin and bradykinin on vasoconstrictor responses evoked by noradrenaline and nerve stimulation. Vasoconstrictor responses (~10 mmHg) were evoked at 5 min intervals by electrical stimulation (6–12 Hz) and in some experiments by noradrenaline injection (0.078–0.156 μg). When three consecutive responses of constant amplitude were obtained, perfusion with angiotensin or bradykinin was commenced. 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.

Effect of captopril or saralasin on the angiotensin-induced potentiation of responses to nerve stimulation. Tissues were exposed to angiotensin I or II as described previously. They were then perfused with Krebs–Ringer solution containing captopril or saralasin. An initial 30 min equilibration period was allowed, during which time electrical stimulation (at 5 min intervals) was continued. The tissues were then perfused with increasing concentrations of angiotensin I or II in the presence of the antagonist. Finally the tissues were perfused with normal Krebs–Ringer solution until stable control responses were achieved.

Drugs used

These were as follows: [Ileu^{5}]angiotensin I (Calbiochem); [Val^{8}]angiotensin II (Hypertensin, Ciba); [Sar^{1}, Ala^{8}]angiotensin II (Saralasin, Beckman); bradykinin tri-acetate (Sigma); captopril (SQ 14 225, Squibb); L-noradrenaline bitartrate (Sigma); phenolamine mesylate (Ciba).

Analysis of results

When the effects of peptide hormones on responses evoked by electrical stimulation or noradrenaline were examined, the control responses in normal Krebs–Ringer solution, before
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and after exposure to the hormone, were measured. Responses evoked in the presence of the hormone were expressed as a percentage change from the mean control value. The results are expressed as means ± SEM. Significant differences (P < 0.05) were evaluated by a paired Student's t-test and by one-way analysis of variance.

Results

Effect of captopril on vasoconstrictor responses evoked by nerve stimulation, by noradrenaline and by potassium chloride

In eight tissues captopril (10−5 mol/l) had no significant effect on the amplitude of vasoconstrictor responses evoked by 4, 8 and 16 Hz electrical stimulation (Fig. 1), but caused a small significant decrease (P < 0.05) in the amplitude of the response evoked by 12 Hz. A higher concentration of captopril (10−4 mol/l, n = 8) caused significant decreases in the amplitude of responses evoked by electrical stimulation at 12 and 16 Hz (P < 0.05, Fig. 1). This effect was more pronounced at 3 × 10−4 mol of captopril/l and was highly significant at 12 and 16 Hz (P < 0.01, Fig. 1).

Captopril at 10−5 mol/l had no significant effect on responses evoked by exogenous noradrenaline (n = 7, Fig. 2). Higher concentrations (1 × 10−4, 3 × 10−4 mol/l) significantly attenuated the vasoconstrictor responses evoked by submaximal doses of noradrenaline (P < 0.05, n = 6 and P < 0.01, n = 6 respectively, Fig. 2). Vasoconstrictor responses, evoked by potassium chloride, were not significantly affected by 3 × 10−4 mol of captopril/l (Fig. 3, n = 6).

Effect of angiotensin and bradykinin on vasoconstrictor responses evoked by nerve stimulation and by noradrenaline

Both angiotensin I and II caused significant dose-related increases in the amplitude of vasoconstrictor responses evoked by nerve stimulation (Figs. 4, 5). Responses evoked by exogenous noradrenaline were also potentiated by angiotensin II. This effect was significantly smaller than the potentiation of responses evoked by electrical stimulation in the same tissues (Table 1). The concentrations of angiotensin I and II used in these experiments had no significant sustained effect on the basal perfusion pressure of the mesenteric vessels. In six tissues bradykinin (8.9 × 10−8 mol/l) had no significant effect on the amplitude of vasoconstrictor responses evoked by electrical stimulation (change in response amplitude −2 ± 2.6%).

Effect of saralasin and captopril on angiotensin-induced potentiation of vasoconstrictor responses

Saralasin (10−8 mol/l) had no effect on the amplitude of responses evoked by electrical stimulation in the absence of exogenous angiotensin (control = 9.0 ± 0.6 mmHg, saralasin
The effects of both angiotensin I and II on vasoconstrictor responses evoked by KCl in mesenteric arteries of the rat were studied. Captopril had no significant effect on the amplitude of vasoconstrictor responses evoked by sympathetic nerve stimulation in rat mesenteric vessels. The magnitude of this potentiation caused by angiotensin was not affected by captopril. The inhibitory effect of low concentrations of the converting enzyme inhibitor significantly reduced the adrenergic-potentiating effect of angiotensin I (Table 2, Fig. 5). However, the potentiation caused by angiotensin II was not affected by captopril.

**Discussion**

The results of the present study indicate that captopril has two potential inhibitory effects at the vascular adrenergic neuro-effector junction. The inhibitory effect of low concentrations of the drug depends on the presence of angiotensin I, in quantities sufficient to potentiate sympathetic vasoconstriction. By contrast, the inhibitory

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**Fig. 3.** Effect of captopril (0, 3 x 10^-4 mol/l, n = 6) on vasoconstrictor responses evoked by KCl in mesenteric arteries of the rat (●, control, n = 6).

**Fig. 4.** Effect of saralasin (10^-8 mol/l) on the angiotensin I- and II-induced potentiation of vasoconstrictor responses evoked by sympathetic nerve stimulation in rat mesenteric vessels. *Increases (%) in response amplitude significantly different from control (P < 0.05). ●, ANG I control, n = 6; ○, ANG I + saralasin, n = 6; ■, ANG II control, n = 6; □, ANG II + saralasin, n = 6).

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**TABLE 1. Effect of angiotensin II on the amplitude of vasoconstrictor responses evoked by noradrenaline and nerve stimulation in mesenteric vessels of the rat**

<table>
<thead>
<tr>
<th>Angiotensin II concn. (mol/l)</th>
<th>Increase in amplitude of vasoconstrictor response (%)</th>
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<tbody>
<tr>
<td>2.9 x 10^-10</td>
<td>+29.0 ± 19.3</td>
</tr>
<tr>
<td>9.7 x 10^-10</td>
<td>+46.0 ± 13.6</td>
</tr>
<tr>
<td>2.9 x 10^-9</td>
<td>+61.0 ± 17.2</td>
</tr>
<tr>
<td>9.7 x 10^-9</td>
<td>+74.0 ± 24.8</td>
</tr>
<tr>
<td>2.9 x 10^-8</td>
<td>+96.0 ± 30.7</td>
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</tbody>
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<tr>
<th>Noradrenaline</th>
<th>Nerve stimulation</th>
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<tr>
<td>+62.0 ± 20.7*</td>
<td>+144.0 ± 32.6*</td>
</tr>
<tr>
<td>+206.0 ± 42.3*</td>
<td>+207.0 ± 32.4*</td>
</tr>
<tr>
<td>+168.0 ± 25.3</td>
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</tbody>
</table>

* % increase in amplitude of response evoked by nerve stimulation significantly greater than % increase in amplitude of noradrenaline evoked response (P < 0.05). The initial amplitude of responses evoked by noradrenaline (13.2 ± 1.6 mmHg) and nerve stimulation (10.8 ± 0.9 mmHg) were not significantly different.

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**Fig. 5.** Effect of captopril (2 x 10^-6 mol/l) on the angiotensin I- and II-induced potentiation of vasoconstrictor responses evoked by sympathetic nerve stimulation in rat mesenteric vessels. *Increase (%) in response amplitude significantly different from control. ●, ANG I control, n = 7; ○, ANG I + captopril, n = 7; ■, ANG II control, n = 5; □, ANG II + captopril, n = 5.
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Table 2. Effect of captopril (6.7 × 10⁻⁸ mol/l) on the angiotensin I-induced potentiation of vasoconstrictor responses evoked by sympathetic nerve stimulation

<table>
<thead>
<tr>
<th>Angiotensin I concn. (mol/l)</th>
<th>Increase in amplitude of vasoconstrictor response (%)</th>
<th>Control</th>
<th>Captopril (6.7 × 10⁻⁸ mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9 × 10⁻⁹</td>
<td>+13.8 ± 8.6</td>
<td>-8.6 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>9.7 × 10⁻⁹</td>
<td>+79.3 ± 15.7</td>
<td>+10.5 ± 9.0*</td>
<td></td>
</tr>
<tr>
<td>2.9 × 10⁻⁸</td>
<td>+170.2 ± 18.8</td>
<td>+48.5 ± 10.4*</td>
<td></td>
</tr>
<tr>
<td>9.7 × 10⁻⁸</td>
<td>+224.8 ± 35.1</td>
<td>+131.3 ± 9.4*</td>
<td></td>
</tr>
<tr>
<td>2.9 × 10⁻⁷</td>
<td>---</td>
<td>+197.0 ± 28.6</td>
<td></td>
</tr>
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</table>

* % increase in response to nerve stimulation in the presence of captopril significantly (P < 0.05) less than in control solution.

The effect of high concentrations of the converting enzyme inhibitor occurs in the absence of exogenous peptide hormones.

Isolated mesenteric vessels, perfused with Krebs-Ringer solution, cannot contain significant amounts of endogenous angiotensin I or II, as saralasin alone had no effect on the responses evoked by electrical stimulation. Therefore, the inhibitory effects of high concentrations of captopril cannot be due to an interaction with endogenous angiotensin. Likewise it cannot be due to elevated levels of endogenous kinins, since bradykinin did not affect the amplitude of responses evoked by nerve stimulation. Since captopril at high concentrations attenuated vasoconstrictor responses evoked by sympathetic nerve stimulation and by exogenous noradrenaline, this inhibitory effect must occur at the vascular smooth muscle cell. Responses evoked by the non-adrenergic stimulant, KCl, were unaffected by captopril. This indicates that the postjunctional inhibitory effect of captopril has some specificity. However, an investigation of the drug’s inhibitory effect on responses evoked by additional vasoconstrictor receptor agonists is required fully to elucidate its nature.

The observation that captopril antagonizes responses to noradrenaline, contrasts with the work of Rubin et al. [12] who were unable to demonstrate an effect of captopril (5 × 10⁻⁴ mol/l) on noradrenaline-evoked contractions of large arteries and veins. Differences in the sensitivity of mesenteric vessels and of large arteries and veins probably account for this discrepancy. It is known that certain α-adrenoceptor antagonists are more potent on mesenteric resistance vessels than on large arteries [10].

The relevance of the postjunctional inhibitory effect of high concentrations of captopril to its therapeutic action in man is unknown. Considering the doses administered, up to 1 g [1], and the relative potency of oral and intravenous doses [12], peak plasma concentrations of 1–2 mg/l (5 × 10⁻⁶-1 × 10⁻⁵ mol/l) are probably. It is therefore unlikely that plasma concentrations of the drug in man could achieve the levels required (10⁻⁴ mol/l) to exert this blocking effect. However, this effect could become significant in animal studies if excessive doses of captopril are given.

Converting enzyme inhibitors alter the plasma levels of angiotensin II and bradykinin in man [13]. The role of the elevated kinin levels in the antihypertensive response to captopril is controversial [14, 15]. Our studies do not allow any conclusion to be reached concerning the role of kinins in the clinical effect of the drug. However, as a high concentration of bradykinin did not affect the vasoconstrictor response to nerve stimulation in the rat mesenteric artery preparation, it is unlikely that this peptide is involved in the effect of captopril on adrenergic reflexes. By contrast, angiotensin II potentiated vasoconstrictor responses evoked by sympathetic nerve stimulation, and to a lesser extent by exogenous noradrenaline. This effect has been reported previously [5, 6] and is clearly not due to a direct constrictor action of the peptide. A number of factors have been proposed to explain this potentiating effect; these include the facilitation of neurotransmitter release, blockade of neuronal uptake and increased sensitivity of the vascular smooth muscle [5, 16]. Since responses to endogenously released noradrenaline (nerve stimulation) were potentiated to a greater extent than those to the exogenous catecholamine, one effect of angiotensin II in this preparation must be the facilitation of neurotransmitter release.

Angiotensin I and II caused similar degrees of potentiation of the responses evoked by electrical stimulation of the sympathetic nerves. As the angiotensin II antagonist, saralasin, displaced the dose–response curves for the two peptides to the same extent, it is likely that angiotensin I is converted into angiotensin II in the mesenteric vessels before exerting its potentiating effect. Low concentrations of captopril blocked this local vascular conversion and selectively antagonized the effect of angiotensin I. Therefore, in situations where endogenous angiotensin levels are sufficient to potentiate adrenergic responses, captopril and other converting enzyme inhibitors will reduce sympathetic vasoconstrictor tone by inhibiting the production of angiotensin II. This could account for the attenuation of reflex and stimulated sympathetic vasoconstriction ob-
served in experimental animals [13, 41] and could contribute to the antihypertensive effect of captopril in man.

Acknowledgments

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References


