Potentiation of Bradykinin by Captopril During Suppression of Prostacyclin Synthesis

ROBERT VANDONGEN, M.D., F.R.A.C.P., ANNE TUNNEY, ANNE BARDEN, B.Sc., and DENIS MAHONEY, B.Sc.

SUMMARY The effect of captopril on blood pressure and on the depressor responses to intravenously administered bradykinin was examined in anesthetized normotensive rats during inhibition of prostaglandin synthesis. The hypotensive action of captopril persisted after treatment with indomethacin in doses that markedly suppressed urinary excretion of the prostacyclin metabolite 6-keto prostaglandin F1α. Captopril markedly potentiated the vasodepressor responses to intravenous bradykinin given by bolus injection or continuous infusion. Neither the magnitude nor the duration of the blood pressure fall were affected by treatment with indomethacin. It is concluded that in the anesthetized rat the hypotensive action of captopril and its augmentation of the depressor response to bradykinin is independent of prostacyclin synthesis. (Hypertension 4: 642-645, 1982)

KEY WORDS • angiotensin converting enzyme • rat blood pressure • indomethacin

Captopril (SQ 14,225) is a potent inhibitor of the peptidyl dipeptide hydrolase, which is responsible for the conversion of angiotensin I (AI) to angiotensin II (AII) and the inactivation of bradykinin during passage of these peptides through the pulmonary vascular bed. In rabbits and rats captopril has increased the magnitude and duration of the hypotensive effect of bradykinin. It has been suggested that the vasodepressor action of bradykinin is mediated by prostaglandins. Recently, bradykinin has been shown to release a prostacyclin-like substance from the dog kidney. The localization of prostacyclin biosynthesis to renal cortical and other vascular sites places it in a unique position to transmit the effect of bradykinin on blood vessel tone.

Methods

Male Wistar rats (average weight, 280 g) consuming a standard commercial diet containing 0.18 mmole Na/g and drinking tap water were anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg). A tracheostomy was performed, and a carotid artery and external jugular vein cannulated with catheters (PE20) filled with heparinized saline. The animals were given heparin (50–100 units i.v.), and mean blood pressure was recorded continuously with a Bell and Howell transducer coupled to a Neurolog recording system (Digitimer, England). When blood pressure was stable, the responses to intravenous bradykinin (Peninsula Laboratories, California) dissolved in saline (0.9 g/100 ml) in 5, 10, 15, and 20 ng bolus doses were determined in five rats. These doses were given again 30 minutes after intraperitoneal administration of captopril (Squibb Institute, Princeton, New Jersey) (3.5 mg/kg).

This study was undertaken to examine the effect of intravenously administered bradykinin on blood pressure in captopril-treated rats after suppression of endogenous prostacyclin synthesis with indomethacin.

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prostacyclin by measuring its chemically stable product 6-keto prostaglandin \( \text{F}_\text{1\alpha} \), using a specific antiserum (Ono Pharmaceuticals, Japan) and \(^{125}\text{I}\) labelled histamine coupled to 6-keto prostaglandin \( \text{F}_\text{1\alpha} \). Free iodinated prostaglandin was separated from the bound form by dextran coated charcoal. In this radioimmunoassay prostaglandin \( \text{D}_\text{2} \) and 6,15-diketo prostaglandin \( \text{F}_\text{1\alpha} \) cross react less than 1%, prostaglandin \( \text{E}_\text{2} \) and prostaglandin \( \text{F}_\text{2\alpha} \) less than 5%. The lower limit of sensitivity of this assay was 15 pg/ml, and the interassay coefficient of variation was 12.3%.

The values between experimental groups were analyzed by the Mann-Whitney method for nonparametric data and within groups by Student’s paired \( t \) test. All values are given as means ± SE.

**Results**

Initial blood pressures in the anesthetized control and indomethacin-treated rats were comparable (table 1). A substantial fall in blood pressure followed the administration of captopril (3.5 mg/kg) in control rats and in rats treated with indomethacin at the two dose levels.

Renal prostacyclin production, assayed as urinary 6-keto prostaglandin \( \text{F}_\text{1\alpha} \), in control rats given phosphate buffer was 266 ± 53 pg/min (n = 8). Mean urine volume was 2.5 ± 0.42 μl/min. Treatment with indomethacin at 3.0 mg/kg reduced this to 27 ± 6 pg/min (n = 9) (\( p < 0.002 \)). Increasing the dose of indomethacin to 6.0 mg/kg reduced this further to 1.7 ± 0.8 pg/min (n = 5) (\( p < 0.004 \), compared with 3.0 mg/kg). Urine volumes were 4.7 ± 2.4 and 1.8 ± 0.6 μl/min after treatment with 3.0 and 6.0 mg/kg indomethacin, respectively.

Bradykinin given intravenously in bolus doses up to 20 ng had no depressor effect before captopril administration (fig. 1). Repeating the injections 30 minutes after captopril administration resulted in an immediate dose-related depressor response (fig. 1). Although the duration of the depressor response varied considerably between rats, blood pressure was usually restored within 1 minute (56 ± 10 seconds after 10 ng bradykinin). Blood pressure was reduced to a similar degree by bradykinin in rats treated with indomethacin at 3.0 and 6.0 mg/kg, which suppressed renal prostaglandin production (fig. 2). The duration of the blood pressure

<table>
<thead>
<tr>
<th>Rat group</th>
<th>No.</th>
<th>Initial MAP (mm Hg)</th>
<th>MAP 30 min after captopril (mm Hg)</th>
<th>MAP 30 min after captopril (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>128 ± 4</td>
<td>105 ± 9*</td>
<td></td>
</tr>
<tr>
<td>Indomethacin-treated</td>
<td>5</td>
<td>131 ± 9</td>
<td>88 ± 11†</td>
<td></td>
</tr>
<tr>
<td>3.0 mg/kg</td>
<td>5</td>
<td>140 ± 6</td>
<td>93 ± 14†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.

\(^* p < 0.05\), for significance of difference from initial values.

\(^\dagger p < 0.01\), for significance of difference from initial values.

**Figure 1.** Mean blood pressure responses to bradykinin in rats (n = 5) before (○—○) and after (●—●) intraperitoneal administration of captopril (3.5 mg/kg).

**Figure 2.** Responses to bradykinin in indomethacin-treated rats (∗ = 3 mg/kg; ▲ = 6 mg/kg) (n = 5) before (□, ○) and after (▲, ●) captopril (3.5 mg/kg).
fall was unaltered by indomethacin at these dose levels (43 ± 8 seconds at 3.0 mg/kg and 52 ± 17 seconds at 6.0 mg/kg after 10 ng bradykinin).

When bradykinin was given by continuous intravenous infusion for 5 minutes (80 ng/kg per min) blood pressure did not change (control group, table 2). However, 30 minutes after administration of captopril, blood pressure dropped within 1 minute of starting the bradykinin infusion. This fall was not maintained, and there was a tendency for blood pressure to increase again by the end of the infusion period. The blood pressure fall seen 30 minutes after captopril administration and subsequently during the infusion of bradykinin was not significantly different in rats treated with indomethacin (3.0 mg/kg) (table 2). When the infusion was stopped after 5 minutes, blood pressure rapidly recovered to preinfusion levels.

**Discussion**

Prostacyclin, which is a potent vasodilator substance synthesized in blood vessels, would be ideally suited to transmit the vasodepressor actions of captopril, although other prostaglandins such as prostaglandin E₂ have also been implicated. However, the blood-pressure-lowering effect of captopril persisted in rats after treatment with doses of indomethacin which substantially reduced urinary excretion of 6-keto prostaglandin F₆ₐ, the stable degradation product of prostacyclin. Since urinary 6-keto prostaglandin F₆ₐ is derived from renal as well as extrarenal degradation of prostacyclin, the assumption is made that its synthesis is equally suppressed in vascular beds where captopril could conceivably act. Although other prostaglandins or metabolites were not measured in this study, inhibition of cyclooxygenase by the dose of indomethacin used would result in comparable suppression, and therefore imply that these substances are also not involved in the hypotensive effect of captopril.

As the assay for 6-keto prostaglandin F₆ₐ was performed on urine without extraction and further separation, the possibility of interference with cross-reacting substances, particularly if these were present in high concentrations, should be considered. However, the marked degree of inhibition achieved is good evidence that the immunoreactive material measured is of prostaglandin origin, if 6-keto prostaglandin F₆ₐ excretion accurately reflects prostacyclin production.

The suggestion that prostaglandins are responsible for potentiating the vasodepressor effect of bradykinin after converting enzyme inhibition in the rabbit and dog is not confirmed by our study in the rat. In these earlier reports, intravenous bradykinin resulted in a biphasic hypotensive response. Indomethacin administration did not alter the immediate initial response but significantly reduced the secondary, sustained hypotensive effect. Such a biphasic response was not seen in the rat, and both the magnitude and duration of the depressor response to bradykinin (which were markedly accentuated by captopril) were unaffected by doses of indomethacin sufficient to suppress prostacyclin synthesis. No depressor responses were observed prior to converting enzyme or kininase II inhibition, indicating rapid pulmonary inactivation of the small doses of bradykinin used.

In conclusion, our findings do not indicate a major role for prostaglandins, particularly prostacyclin, in the blood-pressure-lowering effect of captopril or in the potentiation of bradykinin action in the rat. It is emphasized that variables such as species differences, anesthesia, and the doses of bradykinin used may influence these observations and account for some of the different findings reported by others.

**Acknowledgments**

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**References**

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