Acute ACE Inhibition Causes Plasma Extravasation in Mice That is Mediated by Bradykinin and Substance P

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Abstract—The use of angiotensin-converting enzyme (ACE) has been associated with the occurrence of adverse effects, including cough and angioneurotic edema. Accumulation of kinins has been suggested to play a major role in these adverse effects of ACE inhibitor, although conclusive evidence for such a role is lacking. We investigated whether ACE inhibition increases plasma extravasation in mice (Swiss, C57Bl/6J, and J129Sv/Ev strains) via inhibition of bradykinin metabolism and stimulation of neurogenic inflammatory mechanisms. Intravenous captopril and enalapril increased the extravasation of Evans blue dye in all tissues examined (trachea, stomach, duodenum, and pancreas). This effect was evident 15 minutes after drug administration. The particulate dye Monastral blue identified the sites of captopril-induced leakage in the microvasculature. Pretreatment with the bradykinin B1 receptor antagonist Hoe 140 or with the tachykinin NK1 receptor antagonist SR 140333 inhibited captopril-evoked increase in plasma extravasation. In mice in which the gene encoding the bradykinin B2 receptor was disrupted by gene targeting, neither bradykinin nor captopril increased plasma extravasation. Pretreatment with Hoe 140 did not reduce the hypotensive response induced by captopril. The present findings suggest that ACE inhibition increases kinin levels in tissues and/or plasma. These increased kinin levels increase microvascular leakage in mouse airways and digestive tract via the release of tachykinins from terminals of primary sensory neurons. Exaggerated kinin production and the subsequent stimulation of peptide release from sensory nerves may be involved in adverse effects of ACE inhibitors. (Hypertension. 1998;31:1299-1304.)

Key Words: angiotensin-converting enzyme ▪ captopril ▪ plasma ▪ bradykinin ▪ substance P

Bradykinin and Lys-BK (kallidin) are oligopeptides derived from the enzymatic action of kallikreins on kininogens and are able to promote all the major signs of inflammation, including hyperemia, leakage of plasma proteins, and pain.1–3 Kinins act mainly as local hormones by activating specific receptors, named B1 and B2 receptors, with most of the inflammatory and cardiovascular effects being mediated by the B2 receptor.4 Kinins are powerful algesic agents3 and stimulate a subpopulation of primary sensory neurons to release proinflammatory peptides, including the calcitonin gene–related peptide and the tachykinins SP and NKA.6 This latter effect amplifies the inflammatory response produced by kinins and is referred to as “neurogenic inflammation.” One of the most prominent signs of inflammation caused by kinins and tachykinins is plasma extravasation, an effect that is mediated by B2 receptors and NK1 receptors, respectively.7,8

Kininase II (EC 3.4.15.1) and NEP (EC 3.4.24.11), two dipeptidyl carboxypeptidases, are the major peptidases involved in the catabolism of kinins.9–11 Kininase II is also known as ACE because it causes the conversion of angiotensin I into the vasopressor agent angiotensin II. Both NEP and ACE metabolize tachykinins, and inhibition of NEP and ACE activity has been shown to potentiate the effects of exogenously applied12 or endogenously released kinins and tachykinins.8,13 ACE inhibitors have been widely used in therapy for hypertension, congestive heart failure, and myocardial infarction. Whereas reduced formation of angiotensin II seems to play a major role in the antihypertensive action of ACE inhibitors, increased kinin levels have been proposed to contribute to other beneficial effects of this class of drug, including cardioprotection.14

Therapy with ACE inhibitors is associated with a high prevalence of dry, nonproductive cough and angioneurotic edema.15 Circumstantial evidence supports the view that kinins play a role in these adverse effects of ACE inhibitors, although a variety of observations have led to questioning of this hypothesis.15 In particular, it is not known whether ACE inhibition may cause any inflammatory response and, if so, by which mechanism this response is produced. In the present study, we investigated whether ACE inhibitors are able to increase plasma extravasation in the airways and digestive tract of mice and whether kinins and tachykinins are involved in this effect of ACE inhibitors. We studied plasma extravasa-
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Monastral Blue Extravasation
We used Monastral blue to identify the sites of plasma extravasation in mice. Monastral blue is a colloidal dye that remains within the circulation until gaps form between endothelial cells at sites of inflammation. It then leaves the circulation through gaps between adjacent endothelial cells to become trapped in the basement membrane. Therefore, Monastral blue identifies vessels at the site of plasma extravasation. Monastral blue (30 mg/kg) and captopril (2.5 mg/kg) were coinjected into the left femoral vein. After 30 minutes, mice were transcardially perfused with 200 mL phosphate-buffered saline, gently blotted, and weighed. Half of each tissue was dried by incubation at 60°C for 48 hours and reweighed. Evans blue was quantified as nanograms of Evans blue per milligram of dry weight.

Evans Blue Extravasation
When injected intravenously, the Evans blue dye binds to plasma proteins and thus remains within the vasculature. If plasma extravasation occurs, Evans blue dye leaks out into tissues. Therefore, the Evans blue leak into tissues can be used as a marker for plasma extravasation. Mice were anesthetized with ketamine (50 mg/kg IP) and diazepam (45 mg/kg IP), and the left jugular vein was cannulated. Captopril (5 mg/kg) was administered 25 minutes before Evans blue (30 mg/kg) injection. Five minutes after Evans blue injection, the animals were perfused as described before.

Materials
Xylazine was from Ben Venue Laboratories, and ketamine was from Parke-Davis. Captopril, enalapril maleate, Evans blue, BK, SP, and formamide were from Sigma Chemical Co. Hoe 140 (0-Arg-[Hyp-Thi2,D-Tic-Oic]-Bk) was a generous gift of Dr. K. Wirth (Hoechst), and SR 140333, (5S)-1-2-[3-(3,4-dichlorophenyl)-1-[3-isopropoxyphenylacety]-1-piperidin-3-yl]ethyl)-4-phenyl-1-azoniacyclo(2,2,2), was kindly provided by Dr. X. Emonds-Alt (Sanofi Recherche). Evans blue, captopril, enalapril maleate, BK, SP, and Hoe 140 were dissolved in 0.9% NaCl and the immediate injection of Evans blue caused plasma extravasation. Monastral blue (30 mg/kg) and captopril (2.5 mg/kg) were coinjected into the left femoral vein. After 30 minutes, mice were transcardially perfused with 200 mL phosphate-buffered saline, gently blotted, and weighed. Half of each tissue was dried by incubation at 60°C for 48 hours and reweighed. Evans blue was quantified as nanograms of Evans blue per milligram of dry weight.

Methods
Animals
Male albino Swiss (25 to 30 g, Morini), C57Bl/6J (25 to 30 g, Charles River), J129Sv/Ev (20 to 30 g, Jackson Laboratories), and Bk2r-/- (20 to 30 g, a generous gift of Dr. F. Hess, Merck Research Laboratories) mice and Wistar rats (250 to 300 g, Morini) were used. Bk2r-/- mice were generated by homologous recombination and gene targeting from J129Sv/Ev mice, and stem cells were implanted in C57Bl/6J mouse blastocysts. Animals were housed at a constant temperature (24±1°C) and humidity (60±3%) with a 12-hour light/dark cycle. All procedures complied with the standards for the care and use of animals as stated in Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, Md) and were approved by the local animal care and use committee.

Evans Blue Extravasation
In another series of experiments, mice received the BK2 receptor antagonist Hoe 140 (0.1 mmol/kg IV) or the NK1 receptor antagonist SR 140333 (1 mmol/kg IV), or their vehicles (0.9% NaCl and 5% DMSO, respectively). Captopril (2.5 mg/kg IV) and Evans blue were injected 15 minutes after the antagonists. Five minutes after Evans blue injection, mice were perfused. Evans blue extravasation was also performed in rats. Rats were anesthetized with ketamine (50 mg/kg IP) and diazepam (45 mg/kg IP), and the left jugular vein was cannulated. Captopril (5 mg/kg) was administered 25 minutes before Evans blue (30 mg/kg) injection. Five minutes after Evans blue injection, the animals were perfused as described before.

Statistical Analysis
Each value is the mean±SEM. Statistical analysis was performed using Student’s t test for unpaired data or ANOVA and Bonferroni’s test for multiple simultaneous comparisons. A value of P<0.05 was considered significant.

Results
Effect of Captopril and Enalapril on Evans Blue Extravasation
In the trachea of mice of the Swiss strain, 5 minutes after injection of 0.9% NaCl and the immediate injection of Evans blue caused plasma extravasation.
blue, the dye extravasation was 93 ± 13 ng/mg (n = 6). Intravenous administration of captopril (2.5 mg/kg) 0 or 5 minutes before Evans blue did not affect dye extravasation. However, when captopril was administered 10 or 25 minutes before Evans blue, a significant increase in plasma extravasation was observed (Figure 1, top).

We then studied the effect of increasing doses of captopril injected intravenously 15 minutes before perfusion. A dose of 0.5 mg/kg did not significantly increase the Evans blue dye extravasation in the mouse trachea (Figure 1, bottom). However, higher doses of captopril (1 to 5 mg/kg) caused a significant increase in Evans blue dye extravasation (Figure 1, bottom). Administration of enalapril (1 mg/kg), another ACE inhibitor, also caused a significant increase in plasma extravasation (Figure 1, bottom). Findings similar to those obtained in the trachea were also seen in the stomach, duodenum, and pancreas (data not shown). At 30 minutes after captopril (2.5 mg/kg IV) administration, Evans blue dye extravasation in the rat trachea was higher (98 ± 11 ng/mg, n = 7; P < 0.05) than that seen 30 minutes after vehicle (0.9% NaCl IV) administration (55 ± 9 ng/mg, n = 6). Results similar to those reported in the trachea were observed in the gastric fundus (data not shown).

Monastral blue extravasation was not detected in mice pretreated with the vehicle of captopril (0.9% NaCl, 30 minutes before perfusion) in the trachea and gastric fundus (Figure 2, A and C). In contrast, there was extravasation of Monastral blue from the microvasculature of the trachea and gastric fundus (Figure 2, B and D) after administration of captopril (2.5 mg/kg, 30 minutes before perfusion).

**Studies With Receptor Antagonists and With Bk2r(−/−) Mice**

As already reported in previous studies, pretreatment with the BK B2 receptor antagonist Hoe 140 (0.1 nmol/kg IV) or the tachykinin NK1 receptor antagonist SR 140333 (1 μmol/kg IV) did not affect baseline Evans blue dye extravasation in the mouse trachea, stomach, duodenum, and pancreas (data not shown). Pretreatment with Hoe 140 blocked or markedly reduced the captopril-induced increases

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**Figure 1.** Top, Effect of captopril (2.5 mg/kg IV, shaded columns) or its vehicle (0.9% NaCl, open columns) on Evans blue dye extravasation in mouse trachea (Swiss strain). Bottom, Effect of enalapril and of different doses of captopril (both IV, 15 minutes before perfusion) on Evans blue extravasation in mouse trachea (Swiss strain). Each column is the mean ± SE of at least 5 experiments. *P < 0.05 vs vehicle.

**Figure 2.** Accumulation of Monastral blue in the wall of blood vessels in whole-mount preparations of mouse (Swiss strain) trachea (A and B) or fundus submucosa (C and D) 30 minutes after the injection of captopril (2.5 mg/kg IV, B and D) or its vehicle (0.9% NaCl, A and C). Arrows indicate vessels labeled with Monastral blue. Scale bar = 50 μm.
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Evans Blue Extravasation in Various Mouse Tissues

<table>
<thead>
<tr>
<th>Evans Blue, ng/mg Tissue</th>
<th>Trachea</th>
<th>Pancreas</th>
<th>Stomach</th>
<th>Duodenum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (0.9% NaCl)</td>
<td>107±7</td>
<td>55±7</td>
<td>83±20</td>
<td>132±19</td>
</tr>
<tr>
<td>Captopril (2.5 mg/kg)</td>
<td>250±44*</td>
<td>107±11*</td>
<td>178±19*</td>
<td>257±36*</td>
</tr>
<tr>
<td>Captopril (2.5 mg/kg)</td>
<td>134±12†</td>
<td>57±16†</td>
<td>127±15†</td>
<td>164±19†</td>
</tr>
<tr>
<td>+Hoe 140 (0.1 nmol/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captopril (2.5 mg/kg)</td>
<td>169±24†</td>
<td>71±4†</td>
<td>111±16†</td>
<td>126±12†</td>
</tr>
<tr>
<td>+SR140333 (1 μmol/kg)</td>
<td></td>
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Values are mean±SEM of at least 6 experiments. *P<0.05 vs vehicle; †P<0.05 vs captopril.

in plasma extravasation in trachea, stomach, duodenum, and pancreas (Table). Similarly, SR 140333 caused a remarkable inhibition of the Evans blue dye extravasation in the four tissues examined (Table).

In the trachea of Bk2r−/− mice, baseline Evans blue dye extravasation (98±14, n=5) was not significantly different from that seen in mice of the Swiss strain (Figure 1 and Figure 3). Injection of a dose of BK (10 nmol/kg IV) higher than that which was effective in wild-type mice17 failed to significantly increase plasma extravasation in the trachea of Bk2r−/− mice (Figure 3) and in the duodenum, stomach, and pancreas (data not shown). In contrast, SP (10 nmol/kg IV), which directly acts on NK₁-receptors of postcapillary venules, caused a threefold increase in plasma extravasation in Bk2r−/− mouse trachea (Figure 3). In C57Bl/6 and J129Sv/Ev mice (the mouse strains used to generate Bk2r−/− mice), administration of captopril (2.5 mg/kg IV) significantly increased plasma extravasation in the trachea (Figure 3) and in the other three tissues examined (data not shown).

Direct MBP Measurement

In preliminary experiments, we observed that baseline MBP was not affected by pretreatment with Hoe 140 (1 nmol/kg IV) (data not shown). In anesthetized mice (Swiss strain) pretreated with the vehicle of Hoe 140 (0.9% NaCl IV) and after the injection of the vehicle of captopril (0.9% NaCl IV, control), MBP did not change significantly over time (Figure 4). A significant reduction in MBP was seen 5 minutes after captopril (1 mg/kg IV) administration and throughout the entire period of observation (30 minutes) (Figure 4). The fall in MBP caused by captopril administration was not different in mice pretreated with Hoe 140 (1 nmol/kg IV) or pretreated with its vehicle (0.9% NaCl) (Figure 4).

Discussion

In the present study, we found that acute administration of captopril caused a widespread extravasation of Evans blue dye in the airways, gut, and pancreas of mice. This response was not unique to captopril and was also produced by enalapril. Therefore, this effect is unlikely to be due to a specific chemical property of captopril (for instance, the sulfidryl moiety of this compound); rather, it should be dependent on the ability of this class of drugs to inhibit ACE. Time was a key factor in demonstrating the increase in plasma extravasation caused by ACE inhibitors: a time lag of at least 15 minutes between drug administration and termination of the experiment was required to detect a significant increase in the extravasation of the Evans blue dye. This finding suggests that this effect of captopril was due to one or more peptides that accumulated in a time-dependent manner in plasma and/or tissue. The possibility that the increase in vascular permeability induced by ACE inhibitors was specific to an individual mouse strain (Swiss) was excluded by the
observation that different strains of wild-type mice (C57Bl/6 and J129Sv/Ev) also responded to captopril with significant increases in plasma extravasation. Moreover, the proinflammatory effect of captopril does not seem to be restricted to the mouse. In fact, providing that captopril inhibits ACE for a sufficient time interval (30 minutes), a significant increase in vascular permeability was observed in rat trachea also.

It is well established that the major physiological role of ACE is the conversion of angiotensin I into angiotensin II. However, ACE also inactivates a series of regulatory peptides. Its alternative denomination as kininase II relates to its ability to split the Pro-Phe bond of the nonapeptide BK, thus releasing inactive fragments. There is increasing evidence that administration of ACE inhibitors results in augmented levels of kinins that release vasorelaxant agents such as prostaglandin I2 and nitric oxide. The hypothesis that certain beneficial effects of ACE inhibitors, including cardioprotection, are due to increased kinins at the endothelial level has been advanced. Kinins are proinflammatory peptides. However, an association between ACE inhibition and kinin-dependent “proinflammatory” effects has been reported in only two instances so far: (1) in rat trachea, the combination of captopril with the NEP inhibitor phosphoramidon was reported to increase plasma extravasation; and (2) pretreatment with captopril and another NEP inhibitor, thiophan, caused a contraction in approximately 70% of ferret isolated tracheal strips, and this effect was abolished by different B2 receptor antagonists.

Kinins are the obvious candidate for mediating the plasma protein vascular leakage caused by captopril in the mouse airways and digestive tract. To determine the role of kinins in the plasma extravasation induced by captopril, we used two strategies. First, we blocked BK B2 receptor with Hoe 140, and second, we used Bk2r−/− mice. Pharmacological inhibition or genetic disruption of the BK B2 receptor demonstrated the role of kinins in captopril-evoked plasma extravasation in the four tissues examined. One important proinflammatory mechanism activated by exogenous or endogenous kinins is the release of calcitonin gene–related peptide and of the tachykinins SP and NKA from peripheral endings of a subpopulation of primary sensory neurons. We previously showed that plasma extravasation induced by BK in different mouse tissues was due to the release of SP and NKA from sensory nerve terminals and NK1 receptor activation. In the present experiments, the important contribution of tachykinins to plasma extravasation caused by captopril and mediated by BK is indicated by the marked inhibition of this phenomenon by the NK1 receptor antagonist SR 140333. The present data discriminate clearly between the mechanisms involved in the plasma extravasation and vasodilatation produced by acute captopril administration. In fact, whereas Hoe 140 blocked plasma extravasation, it failed to reduce captopril-induced hypotension. Therefore, at least after bolus administration of captopril in anesthetized mice, it appears that increased kinin levels mediate the increase in microvascular permeability but do not play any important role in the vasodilatory response to captopril.

Although conclusive proof has not been obtained yet, convincing evidence suggests that kinins are produced con-

References


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