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

Costanza Emanueli, Eileen F. Grady, Paolo Madeddu, Michela Figini, Nigel W. Bunnett, Deborah Parisi, Domenico Regoli, Pierangelo Geppetti

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 B2 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

Bradynin 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 extravasation...
Captopril Causes Plasma Extravasation in Mice

Methods

Animals
Male albino Swiss (25 to 30 g, Morini), C57Bl/6J (25 to 30 g, Charles River), IJ295s/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 stem cells were implanted from J129Sv/Ev mice, and gene targeting occurred by selective and high-affinity antagonists of BK B2 and tachykinin NK1 receptors, and we performed experiments in mice in which the gene encoding the BK B2 receptor was disrupted by gene targeting and homologous recombination (Bk2r−/− mice). The site of extravasation was localized by the use of Monastral blue dye in mice, and the effect of the ACE inhibitor captopril on plasma extravasation in the rat tissues was also studied. Finally, the possible role of changes in blood pressure in the plasma protein leakage induced by captopril was investigated.

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 dye leaks into tissues. The Evans blue dye is used as a marker for plasma extravasation. Mice were anesthetized with ketamine (50 mg/kg IM) and xylazine hydrochloride (100 mg/kg IM). To measure intra-arterial MBP, a polyethylene catheter (PE-10 soldered to PE-50, Clay Adams) was inserted into the left carotid artery and advanced into the thoracic aorta. Another PE-10 catheter was inserted into the left jugular vein for injection of drugs. Direct MBP was measured with a Statham transducer (Gould Instruments) connected to the carotid catheter and recorded (Quartet, Basile). Direct MBP was measured under basal conditions for 10 minutes. Hoe 140 (10 nmol/kg) or its vehicle (0.9% NaCl) were then injected, followed 10 minutes later by captopril (1 mg/kg) or its vehicle (0.9% NaCl). MBP was monitored continuously, and values at −10, 0, 5, 10, 15, and 30 minutes from administration of captopril or vehicle were used for statistical analysis.

Materials
Xylazine was from Ben Venue Laboratories, and ketamine was from Parke-Davis. Captopril, enalapril maleate, Evans blue, BK, SP, NEP, 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, (S)-1-{2-[3-[3,4-dichlorophenyl]-1-[3-isoproxyphenoxy]acetyl]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. SR 140333 was dissolved in 5% DMSO solution. In preliminary experiments, we found that the dose of DMSO used in the present experiments (5% IV) had no significant effect on baseline plasma extravasation (data not shown).

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, the dye extravasation was 93 ± 13 ng/mg (n=6). Intra-venous 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<sup>-/-</sup> Mice**

As already reported in previous studies, pretreatment with the BK <sub>B<sub>2</sub></sub> receptor antagonist Hoe 140 (0.1 nmol/kg IV) or the tachykinin NK<sub>1</sub> 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

![Figure 1](http://hyper.ahajournals.org/)

![Figure 2](http://hyper.ahajournals.org/)
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>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>150±11†</td>
<td>55±7</td>
<td>102±15†</td>
<td>164±19†</td>
</tr>
</tbody>
</table>

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<sup>−/−</sup> 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 mice<sup>17</sup> failed to significantly increase plasma extravasation in the trachea of Bk2r<sup>−/−</sup> mice (Figure 3) and in the duodenum, stomach, and pancreas (data not shown). In contrast, SP (10 nmol/kg IV), which directly acts on NK<sub>1</sub>-receptors of postcapillary venules, caused a threefold increase in plasma extravasation in Bk2r<sup>−/−</sup> mouse trachea (Figure 3). In C57Bl/6 and J129Sv/Ev mice (the mouse strains used to generate Bk2r<sup>−/−</sup>) 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 Pro²-Phe² 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, thiopran, 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 continuously by, or in the vicinity of, endothelial cells to exert a protective role on endothelial function. The present data show that at least in mice and rats, ACE inhibition may result in increased kinin levels that cause plasma extravasation. An inflammatory and potentially detrimental effect due to constitutively released kinins is a novel finding of importance on a therapeutic basis. ACE inhibitors are usually well tolerated and are considered relatively safe drugs. However, disturbing or severe adverse effects have been reported, including cough and angioneurotic edema. High incidence (10% to 20%) of dry, nonproductive cough has been described in patients taking ACE inhibitors. Although this type of cough is more troublesome and annoying than disabling, it can be the reason for cessation of the therapy. Various experimental and clinical observations favoring or disfavoring the hypothesis that kinins play a role in ACE inhibitor–induced cough have been provided. More recently, it has been shown that guinea pigs that received captopril in drinking water showed a decrease in the threshold to tussive agents such as citric acid or capsaicin. Although this effect was obtained with a dose of captopril 20 to 40 times higher than doses used in therapeutic regimens, the fact that it was reversed by Hoe 140 suggests that kinins were involved.

Regarding the relevance of the present mouse model to the adverse effects described in humans during ACE inhibitor therapy, it must be underlined that the increase in microvascular permeability in mice was obtained with doses of captopril and enalapril that are similar to those used in clinical settings. It should also be remembered that kinin ability to cause neurogenic inflammation is well documented in rodents, whereas conclusive evidence that this mechanism plays a major pathophysiological role in humans is lacking. However, it is possible that in humans, increased kinin levels after ACE inhibitor administration may contribute to the beneficial drug effect; sensitizing and/or stimulating the airway sensory nerves may cause cough or more severe adverse effects, such as angioneurotic edema. Beneficial or adverse effects of increasing order of severity may depend on individual susceptibility that could be related to kinin levels in plasma and/or tissue. Gender, racial, or genetic differences may identify “susceptible” individuals who are more prone to adverse affects of ACE inhibitors. A recent study showed that in NEP knockout mice, uncontrolled kinin levels mediate increased baseline plasma extravasation in different tissues. It is tempting to speculate that susceptibility to adverse effects of ACE inhibitors may at least in part depend on downregulation of activity of different peptidases involved in kinin and/or tachykinin metabolism.

Acknowledgments
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References


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