Kinin Antagonist Reverses Converting Enzyme Inhibitor–Stimulated Vascular Prostaglandin I₂ Synthesis

William H. Beierwaltes and Oscar A. Carretero

Treatment with a converting enzyme inhibitor has been shown to stimulate aortic prostaglandin I₂ synthesis. We studied whether converting enzyme inhibitor–stimulated prostaglandin I₂ synthesis might be mediated by kinins. Anesthetized male Sprague-Dawley rats were given a continuous 70-minute infusion of either saline or a kinin analogue antagonist, [DArg⁶-Hyp¹-Thr³-Phe⁴-Thr⁷]bradykinin, 8 µg/kg/min. After 10 minutes, rats were given an intravenous bolus of either vehicle or the converting enzyme inhibitor enalaprilat (30 µg/100 g body wt). After 70 minutes, aorta and renal cortical slices were harvested and incubated in vitro in buffer without drugs at pH 7.4, 37°C for 60 minutes. The buffer was then sampled for measurement of 6-keto prostaglandin F₁α (an index of prostaglandin I₂), prostaglandin E₂, and renin release (angiotensin I generation) by radioimmunoassay. The aortic prostaglandin I₂ from rats treated with converting enzyme inhibitor was significantly elevated (36.7±5.0 ng/mg dry wt/hr) compared with aorta from rats treated with either vehicle (25.6±2.2 ng/mg/hr), kinin antagonist (25.1±2.4 ng/mg/hr), or kinin antagonist plus converting enzyme inhibitor (23.0±2.0 ng/mg/hr), p<0.02. There were no differences in aortic prostaglandin E₂, renin release, or prostaglandin E₂ from renal cortical slices. Direct in vitro incubation of aorta with molar concentrations of converting enzyme inhibitor from 10⁻⁹ to 10⁻⁴ had no effect on prostaglandin I₂. These results suggest that kinins may mediate the effect of converting enzyme inhibition on aortic prostaglandin I₂ synthesis and thereby may account for part of the hemodynamic responses resulting from treatment using converting enzyme inhibitors. (Hypertension 1989;13:754–758)

Angiotensin converting enzyme (ACE) inhibitors have been found to be effective therapy for many forms of hypertension. Although designed to inhibit formation of the potent vasoconstrictor angiotensin II, ACE inhibitors are also effective in treatment of patients with apparently renin (angiotensin)-independent hypertension. Likewise, ACE inhibition can lower blood pressure in animal models of hypertension such as the genetic spontaneously hypertensive rat and deoxycorticosterone-induced hypertension where the renin-angiotensin system is not considered a major contributing factor to the elevated blood pressure.

Since ACE is also kininase II, ACE inhibition may lower blood pressure by blocking endogenous kinin degradation when angiotensin is not a critical factor. Scherf et al.⁵ have shown that a nonspecific inhibitor of kallikrein, aprotinin, can attenuate ACE inhibitor–induced aortic prostaglandin (PG) I₂ biosynthesis. Although kinins are vasodilators, these findings suggest they may mediate synthesis of vasodilator prostaglandins. Kinins, whose endogenous degradation is decreased by ACE (kininase II) inhibition, are potent stimulators of phospholipase A₂, the rate-limiting enzyme in the synthesis of prostaglandins. It has been suggested that stimulation of vasodilator PG synthesis by ACE inhibitors may account for their antihypertensive action in renin-independent hypertension. Cyclooxygenase inhibition has been shown to diminish the acute hemodynamic effects of captopril.⁷,⁸

This study will test whether converting enzyme inhibition in vivo leads to altered prostaglandin synthesis in vitro and whether such an effect is mediated by endogenous kinins.

Materials and Methods

Male Sprague-Dawley rats weighing 200–350 g were fasted overnight and were then anesthetized.
with inactin (10 mg/100 g body wt i.p.) (Andrew Lockwood, Inc., Rochester, Minnesota).

**Incubation Protocol.**

The incubation experiments used aortas from untreated normotensive rats. The anesthetized rats were opened by a midventral incision to expose the abdominal aorta. Approximately 2 cm was cleaned in situ and excised; the abdominal aorta was then rinsed of luminal blood in medium, cut into four equal pieces, blotted, and pairs placed in incubation flasks containing 6 ml medium. All experiments were carried out using the bicarbonate buffer medium of Churchill and Churchill, which contains 125 mM NaCl, 19 mM NaHCO₃, 4 mM KCl, 2.6 mM CaCl₂, 1.2 mM NaH₂PO₄, 0.8 mM MgSO₄, and 0.2 g/100 ml glucose. The medium was equilibrated with a 95% O₂ and 5% CO₂ mixture to a pH of 7.4 at a temperature of 37°C. The incubation medium contained 0.1 g/100 ml heat-inactivated bovine serum albumin (BSA) (Difco Laboratories, Detroit, Michigan). The incubation volume (6 ml) was placed in a 25-ml Erlenmeyer flask, continuously gassed with 95% O₂ and 5% CO₂ at 37°C, and shaken at approximately 1 revolution/sec in a Precision shaking water bath (GCA Inc., Chicago, Illinois).

Experiments were carried out by adding either the converting enzyme inhibitor captopril (SQ 14,225, Squibb, Princeton, New Jersey) or enalaprilat (Merck, Sharp & Dohme, Rahway, New Jersey) at molar concentrations of 10⁻⁹, 10⁻⁶, and 10⁻⁴ as well as zero molar (vehicle control). The tissue was incubated under these conditions for 30 minutes, after which time a 1-ml sample was taken for determination of 6-keto PGF₁α, as described below. The incubation continued for another 30 minutes (1 hour total), at which time a second sample was obtained. A third sample was taken for determination of kinins.

**Infusion Protocol.**

The infusion experiments comprised two phases. In the first phase (in vivo), a PE-50 catheter was placed into the carotid artery for a constant infusion of either saline vehicle at 40 μl/min or the kinin analogue antagonist of Stewart and Vavrek (DArg⁵-Hyp¹-Thi⁴-DPhe²-Thi⁵]-bradykinin, Bachem, Torrance, California) at 8 μg/100 g body wt/min over a period of 70 minutes. After the first 10 minutes, a 500-μl bolus of either saline vehicle or 32 μg/100 g body wt of the converting enzyme inhibitor (CEI) enalaprilat was passed into the carotid catheter.

At the conclusion of the infusion period, the rats were opened by a midventral incision to expose the abdominal aorta and kidneys. Approximately 2 cm of the aorta was removed, rinsed in the medium, cut into four pieces, blotted, and two pieces placed into each of two incubation flasks. Results from these paired flasks were combined to give a single mean value representing each rat.

The kidneys were decapsulated, excised, and split longitudinally. One slice was cut from the lateral outer surface of each half using a Stadie-Riggs microtome. The slices were bathed in the medium, blotted, and placed into incubation flasks. The samples were incubated for 60 minutes in 10 ml medium containing heat-inactivated albumin as described above. No drugs were added to any of the incubation flasks during this period. At the conclusion of the incubation period, a 200-μl aliquot was taken for determination of renin concentration and a 1-ml sample taken for prostaglandin determination. All samples were centrifuged and frozen for subsequent analysis. The tissue was removed, oven-dried at 50°C for 24 hours, and weighed. All results are expressed as production over the incubation period factored by milligrams dry weight.

Infusion experiments consisted of four distinct groups: 1) rats receiving a saline infusion and given a saline bolus (NaCl/NaCl); 2) rats receiving a saline infusion but subjected to a bolus of enalaprilat (NaCl/CEI); 3) a third group administered a constant infusion of the kinin analogue antagonist and then given a saline bolus (K-Ant/NaCl); and 4) rats given both an analogue antagonist infusion and an enalaprilat bolus (K-Ant/CEI).

These infusion experiments were repeated using the same four-phase protocol, but replacing enalaprilat with captopril as a bolus (22 μg/100 g body wt) in 500 μl vehicle.

**Analytical Methods.**

Medium renin concentration was determined by radioimmunoassay for generation of angiotensin I (Ang I) based on the method of Haber et al as previously described. Renin concentration is reflected by nanograms Ang I generated per hour of incubation per milliliter of medium. Renin release is corrected for actual volume sampled and dry tissue weight (ng Ang I/hr/mg/incubation time).

Medium prostaglandin concentration was determined by direct radioimmunoassay using Dray’s antibody for PGE₂ (Pasteur Institute, Paris, France) as previously described and Levine’s antibody (Brandeis University, Waltham, Massachusetts) for 6-keto PGF₁α using the method of Levine et al as employed in our laboratory. Each value represents the concentration of PG after 60 minutes of incubation corrected for the dry weight of the tissue sample (pg/mg/hr). All experimental values are compared with concurrent vehicle control experiments. Medium kinin concentration was determined directly by radioimmunoassay as previously described.

All data are presented as the arithmetic mean±1 SEM. The treatment groups were compared by analysis of variance. Changes or differences were considered significant at p<0.05.

**Results.**

Concentrations of 6-keto PGF₁α measured in the tissue incubation medium with various concentra-
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TABLE 1. Concentration-Dependent Response of Aortic Prostacyclin to Incubation With Two Converting Enzyme Inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (M)</th>
<th>0</th>
<th>10^{-9}</th>
<th>10^{-8}</th>
<th>10^{-7}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captopril</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>3.46±0.37</td>
<td>1.80±0.25</td>
<td>2.60±0.27</td>
<td>3.03±0.28</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>4.32±0.53</td>
<td>2.23±0.33</td>
<td>3.39±0.28</td>
<td>3.86±0.33</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td>4.70±0.46</td>
<td>4.96±0.47</td>
<td>4.60±1.06</td>
<td>4.67±1.21</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td>6.41±0.71</td>
<td>6.78±0.60</td>
<td>5.83±1.32</td>
<td>6.91±2.29</td>
</tr>
</tbody>
</table>

Units for 6-keto PGF\textsubscript{1\alpha} (as an index of PGI\textsubscript{2}) in nanograms per milligram dry weight per hour. Concentrations are molar (M). All values mean±1 SEM; n=6 for all values.

Additional experiments were run using captopril (n=9). Aortic 6-keto PGF\textsubscript{1\alpha} in the control (NaCl/NaCl) samples equaled 42.4±9.6 ng/mg/hr. The captopril bolus increased the mean 6-keto PGF\textsubscript{1\alpha} by 40% to 56.9±9.8 ng/mg/hr; however, this increase was not statistically significant. Infusion of the kinin antagonist did not alter 6-keto PGF\textsubscript{1\alpha} compared with the control group (39.2±7.9 ng/mg/hr), and aortas from rats treated with both captopril and the kinin antagonist were also similar to the control group (35.8±7.2 ng/mg/hr).

TABLE 2. Response of Renin and Prostaglandins to Treatment of Rats With the Converting Enzyme Inhibitor Enalaprilat and a Kinin Analogue Antagonist

<table>
<thead>
<tr>
<th>Infusion/Bolus</th>
<th>NaCl/NaCl</th>
<th>NaCl/Enal</th>
<th>K-Ant/NaCl</th>
<th>K-Ant/Enal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical Renin (n=20)</td>
<td>97.7±7.1</td>
<td>119.2±8.1</td>
<td>113.1±10.4</td>
<td>101.4±9.7</td>
</tr>
<tr>
<td>Cortical PGE\textsubscript{2} (n=14)</td>
<td>120±23</td>
<td>160±33</td>
<td>140±20</td>
<td>120±20</td>
</tr>
<tr>
<td>Aortic 6-keto PGF\textsubscript{1\alpha} (n=18)</td>
<td>25.6±2.2</td>
<td>36.7±5.0*</td>
<td>25.1±2.4</td>
<td>23.0±2.0</td>
</tr>
<tr>
<td>Aortic PGE\textsubscript{2} (n=9)</td>
<td>7.4±1.8</td>
<td>7.8±1.5</td>
<td>6.4±1.1</td>
<td>6.3±2.2</td>
</tr>
</tbody>
</table>

All values expressed as mean±1 SEM. Units for renin are nanograms angiotensin I per hour per milligram dry weight per hour and for prostaglandins (PG) are nanograms per milligram dry weight per hour. Enal, enalaprilat; K-Ant, kinin analogue antagonist.

\*p<0.02.
FIGURE 1. Aortic prostaglandin (PG) I$_2$ (as indexed by 6-keto PGF$_{1\alpha}$) and PGE$_2$ production after treatment with a bolus of the converting enzyme inhibitor (CEI) enalaprilat or infusion of a kinin analogue antagonist (K-Ant), or both (n=18). *p<0.02.

is due to ACE/kininase II inhibition rather than some unrelated character of the drug. The captopril data are compromised by a smaller number of observations, and also that captopril (on a molar basis) is considered a somewhat weaker drug. However, the data still suggest that captopril, like enalapril, affects PG synthesis by kininase II inhibition.

Inhibition of ACE/kininase II leads to decreased metabolism of endogenous kinins, and the vascular endothelial cells are a rich source of the enzyme$^{19}$ as well as the primary source of vascular PGI$_2$ synthesis.$^{20}$ Kinins can stimulate phospholipase, and subsequently PG formation. Thus, we hypothesize that ACE inhibition perpetuates endogenous kinin levels, leading to persistent stimulation of PG synthesis. Treatment with captopril results in increased urinary kinin excretion,$^{17}$ while ACE inhibitor-induced aortic PGI$_2$ synthesis can be reversed by aprotinin, a nonspecific inhibitor of kallikrein.$^6$ Although we found that ACE inhibition led to increased aortic PGI$_2$ synthesis, we could completely reverse this stimulation by pretreating our rats with a competitive analogue antagonist of kinins. This seems strong evidence that the effect of ACE inhibition on PG synthesis is mediated through kinins.

We found a weak and insignificant effect of enalaprilat in renal cortex. This does not rule out a renal effect of ACE inhibition on PG synthesis, since the cortex is a site for PG dehydrogenases as well as a variety of kininases. In vivo ACE inhibitor-stimulated renal PG synthesis may be better observed in the PGE$_2$-rich medulla. Several investigators have suggested that ACE inhibitors may act directly in vitro to stimulate PG synthesis. ACE inhibitors added to incubates of aortic tissue$^{6,18}$ or renal medulla$^{21}$ have been reported to result in weak but significant increases in PGI$_1$ or PGE$_2$.

Zusman$^{21}$ has found that the sulfhydryl-containing ACE inhibitor captopril can stimulate PGE$_2$ synthesis in renal medullary cell culture. A captopril analogue that did not inhibit ACE also increased PGE$_2$ synthesis, whereas another analogue devoid of the sulfhydryl group had no effect. Enalapril (with no sulfhydryl group) had no effect on renal medullary PGE$_2$ synthesis. Zusman concluded that captopril somehow stimulated PG synthesis independently from ACE inhibition through some action of the sulfhydryl group. However, several sulfhydryl-free ACE inhibitors have been reported to stimulate PG synthesis, and high concentrations of sulfhydryl-containing ACE inhibitors may inhibit PG synthesis by some mechanism independent of their effect on ACE. Contrary to our results in vivo, our studies employing in vitro incubations of the aorta with captopril and enalaprilat did not show any direct effect of ACE inhibitors on PG synthesis. If we assume kinins mediate the action of ACE inhibitors, then endogenous kinins in vitro would have to be (relatively) increased by such treatments; yet we were unable to find even measurable amounts of kinins in our in vitro preparation. Additionally, kinogen substrate is absent from our blood-free preparation. Zusman$^{21}$ reported finding in vitro stimulation of PGE in the renal medulla by captopril but not enalapril. We have not studied renal medulla.

In summary, our data suggest that enalapril may act in part by stimulating vasodilator PGI$_2$ synthesis, which is reported to be primarily from the vascular endothelial cells where it would be expected to be most important. It is quite possible that this effect is mediated by decreasing degradation of endogenous kinin due to kininase II inhibition, and subsequent kinin stimulation of endothelial phospholipase A$_2$. This mechanism may be important in mediating some of the hemodynamic effects of ACE inhibitors.

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References


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