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

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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, [DArg⁵-Hyp¹-Thi³-Phe⁷-Thi⁸]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)

A ngiotensin 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.¹ Likewise, ACE inhibition can lower blood pressure in animal models of hypertension such as the genetic spontaneously hypertensive rat²–⁴ and deoxycorticosterone-induced hypertension⁵ 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 al⁶ 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,9 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) to the incubation medium in concentrations ranging from 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 Vavrek10 ([D-Arg8-Hyp3-Thi4-dPhe7-Thi8]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 killed 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 al11 as previously described.12 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 l/hr/mg/incubation time).

Medium prostaglandin concentration was determined by direct radioimmunoassay using Dray's antibody for PGE2 (Pasteur Institute, Paris, France) as previously described13,14 and Levine's antibody (Brandeis University, Waltham, Massachusetts) for 6-keto PGF1α using the method of Levine et al15 as employed in our laboratory.13,14 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.16

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

**Results**

**Incubation Protocol**

Concentrations of 6-keto PGF1α measured in the tissue incubation medium with various concentra-
Tables of either captopril or enalaprilat are presented in Table 1. No concentration of either converting enzyme inhibitor significantly increased prostaglandin concentration after either 30 or 60 minutes of incubation. Nor did we find any measurable kinins in the incubation medium of any of the samples.

Infusion Protocol

The results of the four permutations of the infusion protocol with enalaprilat are presented in Table 2. There were no significant differences in the basal renin release from renal cortical slices. The 6-keto PGF₁α from cortical slices was marginally determinable in these samples, and there were no differences between the four parameters. These results are not reported. However, cortical PGE₂ was somewhat (20%), though not significantly, elevated after treatment with enalaprilat.

With incubations of the aorta, enalaprilat produced a significant increase in the 6-keto PGF₁α (Table 2; Figure 1) by some 45%, which was not only greater than incubations from control (NaCl/NaCl) rats, but also greater than rats treated with enalaprilat and the kinin antagonist (p < 0.02). There were no differences between the control group, the group treated with kinin antagonist or enalaprilat and the kinin antagonist. Aortic PGE₂ was not altered by enalaprilat and accounted for only 20% of the total PG measured. In experiments not presented, shorter incubations (15 and 30 minutes) did not result in significant elevations of aortic PG in response to treatment with enalaprilat.

Additional experiments were run using captopril (n=9). Aortic 6-keto PGF₁α in the control (NaCl/NaCl) samples equaled 42.4±9.6 ng/mg/hr. The captopril bolus increased the mean 6-keto PGF₁α 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 PGF₁α 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).

Discussion

Treatment of normotensive rats with the ACE (kininase II) inhibitor enalaprilat in vivo significantly increased production of PGI₂ from harvested aortas in vitro. Furthermore, this response can be completely reversed by pretreatment with a competitive analogue antagonist of kinins.

Several investigators have reported that ACE inhibition may result in increased PG synthesis. Vinci et al17 found that captopril doubled plasma PGE₂, and Dusing et al18 reported that captopril increased PGI₂ synthesis from incubated aortic tissue. Scherf et al16 found that treatment in vivo with ramipril resulted in increased PGI₂ synthesis from aortic strips harvested and incubated in vitro. We have found similar results with enalaprilat and aortic tissue. Also, although our results with captopril were not statistically significant, the trend was toward virtually the same magnitude of change as with enalaprilat, which suggests stimulation of PG synthesis.
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 as well as the primary source of vascular PG synthesis. 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, while ACE inhibitor-induced aortic PG synthesis can be reversed by aprotinin, a nonspecific inhibitor of kallikrein. Although we found that ACE inhibition led to increased aortic PG 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 enalapril 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 PGE2-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 or renal medulla have been reported to result in weak but significant increases in PG synthesis.

Zusman has found that the sulfhydryl-containing ACE inhibitor captopril can stimulate PG synthesis in renal medullary cell culture. A captopril analogue that did not inhibit ACE also increased PG synthesis, whereas another analogue devoid of the sulfhydryl group had no effect. Enalapril (with no sulfhydryl group) had no effect on renal medullary PG 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 renal and aortic PG synthesis in vivo.

In summary, our data suggest that enalapril may act in part by stimulating vasodilator PG 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 A2. This mechanism may be important in mediating some of the hemodynamic effects of ACE inhibitors.

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