Captopril Inhibits Endothelin-1 Secretion From Endothelial Cells Through Bradykinin

Naoko Momose, Keisuke Fukuo, Shigeto Morimoto, and Toshio Oghara

Incubation with captopril, an angiotensin I converting enzyme inhibitor, for 24 hours at concentrations up to 10^{-7} M inhibited endothelin-1 secretion by endothelial cells. This inhibition of endothelin-1 secretion was reversed by coinubcation with 3\times10^{-3} M \(N^o\)-nitro-L-arginine, an inhibitor of nitric oxide synthesis. Furthermore, captopril enhanced the production of nitric oxide in endothelial cells, suggesting that enhancement of nitric oxide production participates in captopril-induced inhibition of endothelin-1 secretion. Moreover, in the presence of 10^{-5} M \(d\)-Arg, \([Hyp^3, Thi^3, D-Phe^7]\) -bradykinin, a bradykinin B_2 receptor antagonist, captopril did not inhibit but rather stimulated endothelin-1 secretion, whereas bradykinin inhibited endothelin-1 secretion, and this inhibition by bradykinin was reversed by coinubcation with \(N^o\)-nitro-L-arginine. In addition, enhancement of nitric oxide production induced by either captopril or bradykinin was inhibited by \(d\)-Arg, \([Hyp^3, Thi^3, D-Phe^7]\)-bradykinin. Although 10^{-4} M des-Arg^9-[Leu]^9-bradykinin, a bradykinin B_2 receptor antagonist, did not affect nitric oxide production by bradykinin, it enhanced the inhibition of endothelin-1 secretion by bradykinin. Furthermore, 10^{-7} M des-Arg^9-bradykinin, a bradykinin B_2 receptor agonist, stimulated endothelin-1 secretion by endothelial cells. These findings suggest that angiotensin I converting enzyme inhibitor inhibits endothelin-1 secretion through the accumulation of endogenous bradykinin in endothelial cells and that the inhibition of endothelin-1 secretion by bradykinin is mediated via B_2 receptors. (Hypertension 1993;21:921-924)

KEY WORDS • angiotensin converting enzyme inhibitors • bradykinin • endothelins • nitric oxide • endothelium

Endothelin-1 (ET-1) is a recently discovered endothelium-derived vasoconstrictive peptide. ET-1 induces a strong and sustained pressor response in vessels and systemic hypertension in the rat. It also stimulates mitogenesis in vascular smooth muscle cells. These studies suggest the potential importance of endothelin in the pathophysiology of hypertension and atherosclerosis. Angiotensin I converting enzyme (ACE) inhibitors generally have been used as antihypertensive drugs. However, the precise mechanism of the antihypertensive effects of ACE inhibitors has not been fully elucidated. In addition, there is recent evidence for new important functions of ACE inhibitors in the cardiovascular field. ACE inhibitors can induce regression of intimal hyperplasia, whereas other antihypertensive drugs are ineffective in this regard. Clinical trials in patients with chronic congestive heart failure demonstrated a significant reduction in mortality with ACE inhibitor therapy. Although ACE inhibition diminishes the formation of a pressor peptide, angiotensin II, it also diminishes degradation of kinins, which are vasodepressor peptides. Bradykinin stimulates the synthesis of prostaglandin I_2 and endothelium-derived relaxing factor, recently identified as nitric oxide (NO). NO not only antagonizes the effects of ET-1 of vasoconstriction and mitogenesis of vascular smooth muscle cells but also inhibits the production of ET-1 by endothelial cells.

Furthermore, ACE inhibitors stimulate the production of NO and prostaglandin I_2 mediated by B_2 receptors in endothelial cells. The present study therefore was performed to examine whether ACE inhibitor inhibits ET-1 secretion through the activation of B_2 receptors in endothelial cells.

Methods

Materials

Captopril, an ACE inhibitor, was donated by Sankyo Co., Tokyo. Bradykinin, \(d\)-Arg, \([Hyp^3, Thi^3, D-Phe^7]\)-bradykinin, des-Arg^9-[Leu]^9-bradykinin, des-Arg^9-bradykinin, and \(N^o\)-nitro-L-arginine (L-NA) were purchased from the Peptide Institute, Osaka, Japan. Nitrate reductase was from Sigma Chemical Co., St. Louis, Mo. Other reagents were of the highest grade available.

Cells

Endothelial cells, isolated from human thoracic aortas, were purchased from Kurabo, Osaka, Japan, and cultured on collagen-coated dishes in modified MCDB 131 medium supplemented with 10% fetal calf serum at 37°C under 5% CO_2 in air. Cells at passages between 5 and 8 were used for the experiments and seeded at a density of 2\times10^4 per well on collagen-coated 24-well plates.

Assay of Endothelin-1

Confluent cells were incubated for 24 hours in serum-free medium containing drugs or vehicle. After incuba-
Hypertension Vol 21, No 6, Part 2 June 1993

Figure 1. Line graph shows dose-dependent effects of bradykinin (BK) on endothelin-1 (ET-1) secretion by endothelial cells. Cells were incubated with serum-free medium containing BK at concentrations indicated for 24 hours in presence (closed triangles) or absence (closed circles) of $3\times10^{-7}$ M N$^\text{G}$-nitro-L-arginine (L-NA). Values are mean±SEM (n=6). *p<0.05, significantly different from control; +p<0.05, significantly different from endothelial cells treated without L-NA.

Figure 2. Line graph shows dose-dependent effects of captopril on endothelin-1 (ET-1) secretion by endothelial cells. Cells were incubated with serum-free medium containing captopril at concentrations indicated for 24 hours in presence (closed triangles) or absence (closed circles) of $3\times10^{-7}$ M N$^\text{G}$-nitro-L-arginine (L-NA). Values are mean±SEM (n=6). *p<0.05, significantly different from control; +p<0.05, significantly different from cells treated without L-NA.

Results

After 24 hours of incubation of endothelial cells with serum-free medium, ET-1 concentration in the control culture was 1,321±42 pg/mg cell protein (n=6). Treatment of endothelial cells with bradykinin at concentrations from $10^{-7}$ to $10^{-5}$ M significantly inhibited this basal secretion of ET-1 (Figure 1). This inhibition by bradykinin was partially reversed by coincubation with $3\times10^{-3}$ M L-NA, an inhibitor of NO synthesis, at a higher concentration than that of L-arginine in the medium ($3\times10^{-4}$ M).

Incubation of endothelial cells with captopril at concentrations of $10^{-8}$ and $10^{-7}$ M significantly suppressed ET-1 secretion to the same degree as incubation with bradykinin (Figure 2). Coincubation with $3\times10^{-3}$ M L-NA partially reversed this suppression by captopril of the endothelial cells. On the other hand, in the presence of $10^{-4}$ M d-Arg$_2$[Hyp$_3$,Thi$_8$,d-Phe$_7$]-bradykinin, a B$_2$ receptor antagonist, $10^{-7}$ M captopril did not inhibit but rather stimulated ET-1 secretion (Figure 3).

Although $10^{-7}$ M bradykinin inhibited ET-1 secretion, this inhibition by bradykinin was significantly enhanced...
by coincubation with 10⁻⁶ M des-Arg⁶-[Leu⁷]-bradykinin, a B₁ receptor antagonist. Furthermore, 10⁻⁷ M des-Arg⁶-bradykinin (a B₁ receptor agonist) stimulated ET-1 secretion by endothelial cells (Figure 3).

We next examined whether captopril enhances NO production in cultured endothelial cells. As shown in Figure 4, 10⁻⁷ M captopril significantly enhanced NO production by endothelial cells. The B₁ receptor antagonist at 10⁻⁴ M did not affect this enhancement of NO production by captopril. In contrast, the B₂ receptor antagonist at 10⁻⁴ M attenuated this enhancement of NO production by captopril. Although 10⁻⁷ M bradykinin significantly enhanced NO production, the B₁ receptor agonist at 10⁻⁷ M did not affect NO production. Furthermore, bradykinin-induced NO production was completely inhibited by 10⁻⁶ M B₁ receptor antagonist but not by 10⁻⁶ M B₂ receptor antagonist.

**Discussion**

The present study demonstrates that the ACE inhibitor captopril inhibits the basal secretion of ET-1 by vascular endothelial cells. Coincubation with L-NA, an inhibitor of NO synthesis, reversed the inhibition by captopril. Furthermore, captopril actually enhanced NO production in endothelial cells, suggesting that NO participates in this inhibition of ET-1 secretion by captopril.

Recently, Yoshida and Nakamura also reported that ACE inhibitors inhibit 5% calf serum-stimulated ET-1 secretion but not the basal secretion of ET-1 by endothelial cells. However, they did not consider the participation of bradykinin or NO in the cellular mechanism of this inhibitory effect of ACE inhibitors. ACE inhibition decreases not only formation of angiotensin II⁸ but also degradation of kinins. In cultured human and bovine endothelial cells, ACE inhibitors elevate intracellular calcium concentrations and stimulate the production of NO and prostaglandin I₂ via B₂ receptors. In the present study, the enhancement of NO production by captopril was inhibited by coincubation with the B₂ receptor antagonist. In addition, our preliminary experiments revealed that the concentration of bradykinin in the medium, determined by a specific radioimmunoassay kit (Otsuka Pharmaceutical Co. Ltd.), increased from 0.08±0.01 to 3.17±0.14 ng/mL (mean±SEM, n=6) after 24 hours of incubation of endothelial cells with 10⁻⁷ M captopril. Although the medium concentration of bradykinin in 10⁻⁷ M captopril-treated cells was lower than that of exogenous bradykinin, which inhibited ET-1 secretion to the same degree as incubation with 10⁻⁷ M captopril, it has been reported that inhibition of bradykinin degradation by captopril potentiates the vasodilator effect of bradykinin in rat mesenteric arteries. Furthermore, ACE inhibition by ramiprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium-derived bradykinin. Therefore, accumulation of endogenous bradykinin by the inhibition of bradykinin degradation could be responsible for the inhibition of ET-1 secretion by captopril from endothelial cells.

Bradykinin, produced from kininogen by the action of kallikrein, has a variety of physiological and pharmacological effects on the vascular system; physiologically it induces endothelium-dependent relaxation of arteries. However, it also induces contraction of arteries and veins. These different effects of bradykinin on arteries have been explained by the existence of bradykinin receptor subtypes. Endothelial cells possess low-affinity B₁ receptors and high-affinity B₂ receptors. In this study, bradykinin-induced stimulation of NO production was inhibited by the B₂ receptor antagonist but not by the B₁ receptor antagonist. Because bradykinin-induced suppression of ET-1 secretion was reversed by L-NA, enhancement of NO production via the B₂ receptor may participate in the suppression of ET-1 secretion by bradykinin. In contrast, coincubation with B₁ receptor antagonist did not reverse but rather enhanced the bradykinin-induced suppression of ET-1 secretion. Furthermore, the B₁ receptor agonist did not affect NO production but significantly stimulated ET-1 secretion by the endothelial cells. Thus, although bradykinin physiologically inhibits ET-1 secretion by enhancing NO production via B₂ receptors, bradykinin can stimulate ET-1 secretion when B₁ receptor pathways are more activated than B₂ receptor pathways in endothelial cells. In addition, the enhancement of NO production by captopril was inhibited by B₂ receptor antagonist but not by B₁ receptor antagonist. Furthermore, although captopril alone inhibited the secretion of ET-1, it stimulated ET-1 secretion when captopril-induced enhancement of NO production was inhibited by coincubation with B₂ receptor antagonist. These results suggest that captopril suppresses ET-1 secretion by the accumulation of endogenous bradykinin in endothelial cells under physiological conditions. However, captopril can stimulate ET-1 secretion under pathological conditions in which endothelial cells possess predominantly B₁ receptors rather than B₂ receptors.
Acknowledgments

We are grateful to Taeko Kaimoto for technical assistance and Chiaki Murakami for secretarial work.

References


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Hypertension. 1993;21:921-924
doi: 10.1161/01.HYP.21.6.921

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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World Wide Web at:
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