Captopril Increases the Affinity of Bradykinin Receptor Binding Sites in Bovine Coronary Arterial Endothelial Cells

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ABSTRACT—In a radioligand binding study using bovine coronary artery endothelial cell membranes, captopril changed a single bradykinin (BK) binding site (Kd = 1.77 nM, Bmax = 60.2 fmol/mg protein) to high- (Kd = 0.68 pM, Bmax = 17.7 fmol/mg protein) and low- (Kd = 1.00 nM, Bmax = 72.5 fmol/mg protein) affinity binding sites. This effect was reversed by GppNHp. Captopril also enhanced BK-induced endothelium-dependent relaxation in saponin-treated coronary rings, and GppNHp partially suppressed this enhancement. These results suggest that captopril may affect BK receptors that couple to G-proteins.

Keywords: Bradykinin, Captopril, Coronary artery

Bradykinin (BK) induces endothelium-dependent relaxation of coronary arteries isolated from pigs (1), dogs (2), humans (3) and cattle (4, 5). Captopril, an angiotensin (AT) I converting enzyme (ACE) inhibitor, enhances this relaxation (5). The potentiation of BK by captopril may be explained by the following mechanism: i) inhibition of BK degradation, ii) a shift in AT I metabolism towards relaxant AT derivatives, and iii) allosteric facilitation of BK receptor activation (6–8). Similarly, an ACE inhibitor affected β-adrenoceptors at the binding sites (9). However, although inhibition of BK degradation might be involved in the action of captopril (6), other (as yet unknown) mechanisms may also be involved because ACE-resistant BK analogs are equally as well potentiated by ACE inhibitors (8). Since there have been no reports regarding the effect of captopril on the affinity of BK for, and binding to, its receptor, we investigated their interaction in a radioligand binding study. To clarify the interaction between BK receptor and G-proteins, GppNHp (a non-hydrolyzable analogue of GTP) was used in the presence of captopril. A functional study was then performed to confirm the results of the binding study.

Coronary arteries from freshly slaughtered cattle (castrated male, Japanese black beef cattle, about 2.5-year-old) were obtained from an abattoir and transferred to our laboratory immersed in ice-cold Krebs-Ringer bicarbonate solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, 25 mM NaHCO3, 1.2 mM KH2PO4 and 10 mM glucose), aerated with 95% O2 and 5% CO2. The left circumflex arteries were cut longitudinally and the endothelial cells were collected in 10 ml ice-cold lysis buffer (0.1 mM phenylmethylsulfonyl fluoride, 5 mM EDTA, 5 mM EGTA, 100 mM NaCl and 5 mM Tris HCl, pH 7.4) by a single gentle scrape with a surgical blade (no. 10; Feather Kogyo, Tokyo). Coronary artery endothelial cell membranes were prepared as described previously (11). The protein concentration of the final suspension was measured by the method of Lowry et al. (12). Aliquots (approximately 100 μg protein) of the membrane fraction were incubated with various concentrations of [3H]BK. After 60 min of incubation at 25°C, membrane-bound and unbound ligand was separated by rapid filtration through a glass fiber filter (GF/C; Whatman, Maidstone, UK). The tissue-bound radioactivity was extracted from the filters into scintillation fluid and counted using a liquid scintillation counter (LSC-3050; Aloka Co., Tokyo). The specific binding of [3H]BK was defined as the difference between binding in the absence and presence of 10 μM unlabeled BK. In another experiment, aliquots of the membrane fraction were incubated in the presence of 10 μM B1- or B2-kinin receptor antagonists, together with 0.05 or 5 nM [3H]BK. The dissociation constant (Kd) and the maximum binding capacity (Bmax) were calculated using the EBDA or LIGAND computer program (13).

For the functional study, the coronary artery was cut into 3–4-mm rings, which were mounted horizontally in an organ bath filled with 5 ml Krebs-Ringer bicarbonate solution. The solution was maintained at 37°C, aerated with 95% O2 and 5% CO2, and had a final pH of 7.4. Each coronary artery ring was attached to a force transducer (TB-611T; Nihon Kohden Kogyo, Tokyo), and isometric force was recorded on a pen recorder (WI-641G, Nihon Kohden.
Kogyo). The ring was stretched to an optimal tension of 20 mN and allowed to equilibrate for 90–120 min before starting the experiment. All experiments were performed in the presence of indomethacin (10 μM) to eliminate the effects of prostanooids. The ring was precontracted with 3 μM prostaglandin F₂₀ (PGF₂₀) before adding BK. The presence of the endothelium was verified by inducing relaxation with acetylcholine.

In the measurement of ACE activity, the left circumflex arteries were cut into an approximately 10 × 10 mm square, and activity was measured using the method of Cushman and Cheung (10) and expressed as picomoles of hippuryl-histidyl-leucine hydrolyzed per minute per surface area of endothelium (pmol/mm² per minute).

The following drugs were used: saponin (Merck, Darmstadt, Germany); PGF₂₀ (Ono Pharmaceutical Co., Ltd., Osaka); indomethacin, sodium nitroprusside (Nacalai Tesque, Inc., Kyoto); captopril (Research Biochemical Incorporated, Natick, MA, USA); phenylmethylsulfonyl fluoride, bradykinin, des-Arg⁹, [Leu⁸]-BK, [Thr⁵⁸, d-Phe¹⁵]-BK, hippuryl-histidyl-leucine, histidyl-leucine and GppNHp (Sigma Chemical Co., St. Louis, MO, USA); [2,3-prolyl-3,4-²H(N)]BK (specific activity, 62.0 Ci/mmol; New England Nuclear, Waltham, DE, USA).

All results are expressed as mean values ± S.E.M. Statistical analyses were performed by Student’s t-test or Tukey’s test after one-way (in ACE activity) and two-way (in functional study) analyses of variance, with a significance threshold of 5%.

Figure 1 shows the specific binding of [³H]BK to the membrane fraction of bovine coronary artery endothelial cells and the resulting Scatchard plot. The specific binding appeared to be saturable. The Kᵅ value was calculated as 1.77 ± 0.99 nM and Bₘₐₓ was 60.2 ± 3.5 fmol/mg protein. The Scatchard plot yielded a single line, and the Hill coefficient was 0.95 ± 0.04, which was not significantly different from unity. These results suggest that [³H]BK binds to a single class of noncooperative site. The pKᵅ value (8.8) for [³H]BK was similar to the pD₂ value (9.0) for BK estimated from our previous functional study (5). In the presence of captopril (10 μM), specific [³H]BK binding was saturable and occurred at high- and low-affinity binding sites. The Scatchard plot also indicated the presence of high- and low-affinity binding sites. The Kₛ and Bₘₐₓ values calculated for the high-affinity site were 0.68 ± 0.28 PM and 17.7 ± 2.1 fmol/mg protein, while those for the low-affinity site were 1.00 ± 0.20 nM and 72.5 ± 11.3 fmol/mg protein. The Hill coefficient was 0.64 ± 0.11, which was significantly less than unity. Co-addition of GppNHp with captopril resulted in loss of the high-affinity binding site, indicated by the return of the Scatchard plot to a single line. Under these conditions, the Kₛ and Bₘₐₓ values were 2.03 ± 1.16 nM and 58.1 ± 8.3 fmol/mg protein, and the Hill coefficient was 0.95 ± 0.06, which was not significantly different from unity. A B₂-receptor antagonist, [Thr⁵⁸, d-Phe¹⁵]-BK, inhibited most of both types of affinity site; however, a B₁-receptor antagonist, des-Arg⁹, [Leu⁸]-BK, did not (Fig. 2c). Thus, as demonstrated in our previous functional study (5), the B₂-receptor appears to be involved in both affinity binding sites. Captopril might therefore induce high-affinity binding sites by changing the conformation of the B₂-receptor. These high-affinity binding sites, which are affected by GppNHp, appear to be G-protein-coupled B₂-receptor receptors. GppNHp has been suggested to have a similar effect on B₂-receptor receptors in bovine aorta endothelial cells (14).

To confirm these results, a functional study was performed using bovine coronary artery rings with intact endothelium. BK evoked concentration-dependent relaxation of rings that had been precontracted with 3 μM PGF₂₀, but no relaxation in endothelium-denuded arteries (data not shown). To increase endothelial permeability to GppNHp, the intact coronary artery was treated with 0.002% saponin for 10 min. This concentration and treatment time was chosen to optimize permeabilization of the endothelial cell membrane and minimize damage to the smooth muscle cell.
membrane. There were no changes in contractions induced by 60 mM KCl and 3 μM PGE\textsubscript{2} before and after saponin treatment. Maximal BK-induced relaxation was inhibited by 84% after saponin treatment. As shown in Fig. 3, captopril (10 μM) enhanced BK-induced relaxation in the saponin-treated coronary artery, decreasing the EC\textsubscript{50} value from 1.55 ± 0.23 nM to 0.13 ± 0.03 pM. GppNHp (10 μM) partially inhibited this captopril-induced enhancement of BK relaxation, but had no significant effect without saponin pretreatment (data not shown). These results suggest that captopril increases the affinity of BK for B\textsubscript{2}-kinin receptors and that addition of GppNHp decreases its affinity. One of the reasons why the inhibitory effect of GppNHp was partial might be due to the insufficient permeability of GppNHp into the endothelial cells.

Captopril at 10 μM was enough to inhibit the ACE activity in bovine coronary artery. ACE activity in the presence of captopril was as follows: captopril at 10 μM, 1.32 ± 0.52**; 0.1 μM, 3.41 ± 2.67**; 1 nM, 5.98 ± 0.50*; control, 8.74 ± 1.48 pmol/ml/mm\textsuperscript{2} per minute (*P<0.05, **P<0.01 vs control).

The potentiating effects of captopril on BK binding affinity, together with the relaxation of intact coronary arteries produced by BK and its inhibition by GppNHp, suggest that B\textsubscript{2}-kinin receptors might be allosterically regulated by ACE activity as well as G-proteins. A similar possibility was suggested by Auch-Schwelk et al. (15), using another ACE inhibitor, lisinopril. However, further studies are needed to clarify whether these effects of captopril depend on inhibition of ACE activity or others. We did not investigate whether captopril shifted AT I metabolism towards the relaxant AT-(1-7) derivative, which stimulates NO formation in vascular endothelium. Further studies will therefore be needed to clarify this point.

The present results suggest that captopril might enhance BK-induced arterial relaxation by increasing the affinity of BK binding sites on B\textsubscript{2}-kinin receptors that couple to G-proteins.

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