Endothelium-Dependent Relaxation in Response to Low Concentrations of Bradykinin Is Enhanced by Phosphoramidon, Bosentan and BQ-123 in Bovine Coronary Arteries In Vitro

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ABSTRACT—An endothelin (ET)-converting enzyme inhibitor phosphoramidon (10 \( \mu \)M), an ET\(_{AB}\)-receptor antagonist bosentan (10 \( \mu \)M) and an ET\(_A\)-receptor antagonist BQ-123 (1 \( \mu \)M) potentiated endothelium-dependent relaxation of bovine coronary arteries in response to bradykinin (BK) at femtomolar to picomolar concentrations, but not at nanomolar concentrations. BQ-788 (3 \( \mu \)M), an ET\(_B\)-receptor antagonist, showed no significant effects on fM – nM BK-induced relaxation. These results suggest that the endothelium-dependent relaxation of isolated bovine coronary arteries induced by very low concentrations of BK is partly regulated by a complex mechanism involving the ET\(_A\)-receptor antagonism.

Keywords: Bradykinin, Endothelin, Endothelium-dependent relaxation

It is well known that endothelial cells play a key role in the regulation of vascular tone through release of vasodilating and vasoconstricting substances such as NO and endothelin (ET) in response to alterations in hemodynamic factors (1). Antagonism between NO and ET-1 has been demonstrated in human arteries (2).

It has been reported that bradykinin (BK) induces endothelium-dependent relaxation of arteries isolated from a variety of species by releasing endothelium-derived relaxing factors (1). Our previous study showed that very low concentrations of BK induced relaxation which was mediated by NO through stimulation of B\(_2\)-receptors on the endothelium in isolated bovine coronary arteries (3). Recently we found that phosphoramidon (4, 5), an ET-converting enzyme inhibitor, enhanced the endothelium-dependent relaxation evoked by very low concentrations of BK (6). Moreover it has been reported that BK suppresses ET-induced contraction of arteries through its B\(_2\)-receptor on the endothelium (7). The present study was done to determine whether or not ET-1 participates in the relaxant response to very low concentrations of BK using ET-1 antagonists.

Experiments were performed on circumflex coronary arteries isolated from bovine hearts obtained from a slaughterhouse. Each coronary artery was carefully dissected out and cleaned of adhering connective tissue, and two rings were cut about 3 mm. Each ring was mounted horizontally in an organ bath filled with 15 ml of Krebs-Ringer bicarbonate solution of the following composition: 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl\(_2\), 1.2 mM KH\(_2\)PO\(_4\), 2.5 mM CaCl\(_2\), 25 mM NaHCO\(_3\), and 10 mM glucose. The bath solution was maintained at 37°C and gassed with 95% O\(_2\) and 5% CO\(_2\). The coronary artery ring was attached to a force transducer (TB-611T; Nihon Kohden Kogyo Co., Tokyo), and isometric force was recorded on a pen recorder (WI-641G, Nihon Kohden Kogyo Co.). Each ring was stretched to an optimal tension of 20 milliNewtons, as determined by repeated stimulation with 60 mM KCl. Each ring was allowed to equilibrate for 90 – 120 min before starting the experiment. All experiments were performed in the presence of 10 \( \mu \)M indomethacin to eliminate the effects of prostanoids (8). The ring was contracted with 3 \( \mu \)M prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)) before the addition of BK. Cumulative concentration-response curves were obtained by increasing the concentration of BK in a cumulative manner; the addition was made as soon as a steady response had been obtained with the preceding concentration. When an ET converting enzyme inhibitor or ET-antagonists were used, each drug was added to the organ bath 30 min before the concentration-response curves for BK were obtained. In coronary rings contracted with PGF\(_{2\alpha}\), the responses were expressed as a percentage of the relaxation induced by 100 \( \mu \)M sodium nitroprusside (SNP). Unless stated other-
wise, n refers to the number of animals from which the coronary rings were isolated. The results were expressed as mean values ± S.E.M. Statistical evaluation of the data was performed using Student’s paired t-test. Significance was established when the probability level was less than 5%.

BK (Sigma Chemical Co., St. Louis, MO, USA), indomethacin and SNP (Nacalai Tesque, Kyoto), PGF$_2$ (Ono Pharmaceutical Co., Ltd., Osaka), ET-1 and phosphoramidon (Peptide Institute Inc., Osaka) were obtained commercially. Bosentan, BQ-123 (cyclo-(D-Trp-D-Asp-Pro-D-Val-Leu)-Na) and BQ-788 (N-cis-2,6-dimethylpiperidino-carbonyl-L-$\gamma$-methylleucyl-D-$\text{N}^\text{cis}$-methoxybenzyltryptophanyl-D-norleucine) were donated by Nippon Roche Pharmaceutical Co., Ltd. (Kamakura) and Banyu Pharmaceutical Co., Ltd. (Tsukuba), respectively.

Endothelium-dependent relaxant responses to very low concentrations (10–100 pM) of BK were significantly enhanced by phosphoramidon (Fig. 1a). It has been reported that phosphoramidon, a metalloproteinase inhibitor (4), has inhibitory effects on a neutral endopeptidase (9) that catalyzes the degradation of BK and on an ET-converting enzyme (5) that catalyzes the conversion of big ET-1 to ET-1 (5). In this experiment, we examined one possibility that ET-converting enzyme inhibition might contribute to the potentiating effect of phosphoramidon on the relaxation induced by very low concentrations of BK. Firstly, the ef-

![Fig. 1. Effects of phosphoramidon (a), bosentan (b), BQ-123 (c) and BQ-788 (d) on bradykinin (BK)-induced endothelium-dependent relaxation of isolated bovine coronary arteries precontracted with PGF$_2$ (3 μM) in the presence of indomethacin (10 μM). Relaxation induced by sodium nitroprusside (100 μM) was taken as 100%. Each point represents the mean ± S.E.M. of eight coronary rings obtained from different animals. Closed circle, control; open circle, drug-treated. *Significantly different from the corresponding control values (*P<0.05, **P<0.01).](image-url)
fects of some ET-1 antagonists on the relaxation induced by BK were examined. An ET$_{A}$-receptor antagonist, bosentan (10), and an ET$_{A}$-receptor antagonist, BQ-123 (11), enhanced the relaxation induced by very low concentrations of BK (Fig. 1: b and c). However, the ET$_{B}$-receptor antagonist BQ-788 (12) had no significant effect on the relaxation induced by very low concentrations of BK (Fig. 1d).

Phosphoramidon (10 $\mu$M), bosentan (10 $\mu$M), BQ-123 (1 $\mu$M) and BQ-788 (3 $\mu$M) showed no significant effects on vascular tone under both resting and PGF$_{2\alpha}$-induced conditions.

This is the first report to show that BK-induced relaxation is enhanced by an ET-converting enzyme inhibitor and by ET-antagonists. These results suggest that the relaxation induced by low concentration of BK in bovine coronary arteries is regulated by NO and ET-1 from endothelial cells (1) and that the released ET-1 contracts the smooth muscle cells upon stimulation of ET$_{A}$-receptors (S. Ishiguro et al., unpublished data). Our preliminary data showed that the bovine coronary rings with endothelium released spontaneously NO (pM level) and ET-1 (fM level) and that BK (10$^{-12}$ and 10$^{-8}$ M) concentration-dependently enhanced the release of NO and inhibited the release of ET-1 from these rings (A. Nishio et al., unpublished data). Further studies are needed to clarify that ET-1 at fM level affects the BK-induced relaxation in this artery. The relaxation induced by relatively high concentrations of BK (above 100 nM) was not enhanced by phosphoramidon, bosentan or BQ-123. The precise reasons for this are unclear. One possibility is that a relatively high concentration of BK releases much more NO from endothelial cells, and that NO inhibits the formation or release of ET from endothelial cells (13, 14).

In conclusion, the present findings suggest that the endothelium-dependent relaxation of isolated bovine coronary arteries induced by very low concentrations of BK is partly regulated by a complex mechanism involving the ET$_{A}$-receptor antagonism.

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REFERENCES