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Stimulation of bradykinin B_2-receptors on endothelial cells induces relaxation and contraction in porcine basilar artery *in vitro*

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1 The aim of the present study was to characterize the subtypes of bradykinin (BK) receptors that evoke the relaxation and contraction induced by BK and to identify the main contracting and relaxing factors in isolated porcine basilar artery by measuring changes in isometric tension and a thromboxane (TX) metabolite.

2 Endothelial denudation completely abolished both responses. [Thi^{5,8}, D-Phe^{7}]-BK (a B_2-receptor antagonist) inhibited the BK-induced relaxation and contraction, whereas des-Arg^{9}, [Leu^{8}]-BK (a B_1-receptor antagonist) had no effect.

3 L-nitro-arginine (L-NA, a nitric oxide synthase inhibitor) completely inhibited BK-induced relaxation. Indomethacin (a cyclo-oxygenase inhibitor) completely and ONO-3708 (a TXA_2/prostaglandin H_2 receptor antagonist) partially inhibited BK-induced contraction, whereas OKY-046 (a TXA_2 synthase inhibitor) and nordihydroguaiaretic acid (a lipoxygenase inhibitor) did not.

4 In the presence of L-NA, the contractile response to BK was inhibited by indomethacin or ONO-3708 and was competitively antagonized by [Thi^{5,8}, D-Phe^{7}]-BK ($pA_2 = 7.50$). In the presence of indomethacin, the relaxant response to BK was inhibited by L-NA and was competitively antagonized by [Thi^{5,8}, D-Phe^{7}]-BK ($pA_2 = 7.59$).

5 TXA_2 release was not induced by BK-stimulation.

6 These results suggest that the endothelium-dependent relaxation and contraction to BK in the porcine basilar artery is mediated *via* activation of endothelial B_2-receptors. The main relaxing factor may be NO and the main contracting factor may be prostaglandin H_2.

Keywords: Bradykinin; B_2-receptor; cerebral artery; endothelium; nitric oxide; prostaglandin

Abbreviations: BK, bradykinin; EDCF, endothelium-derived contracting factor; L-NA, L-nitro-arginine; NDGA, nordihydroguaiaretic acid; NO, nitric oxide; TX, thromboxane; OKY-046, sodium (E)-3[4-(*l*-imidazoly-methyl)phenyl]2-propenoate; ONO-3708, L-arginine salt of 9,11-dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-(β)-cyclopentyl-ω-pentanor-TXA_2; PG, prostaglandin; UTP, uridine 5′-triphosphate

Introduction

Species differences in the *in vitro* responsiveness of basilar arteries to bradykinin (BK) have been reported. Basilar artery from humans (Whalley *et al*., 1987; Hatake *et al*., 1990; Onoue *et al*., 1994) responds to BK with relaxation, that from guinea-pigs (Edvinsson *et al*., 1990) responds with contraction, and those from rats (Nishimura & Suzuki, 1997), rabbits (Whalley *et al*., 1983) and dogs (Tsuji & Cook, 1995) respond with relaxation and contraction. Cat (Whalley & Wahl, 1983) and bovine (Miyamoto *et al*., 1994) basilar arteries do not respond to BK. The actions of BK have been reported to be mediated *via* activation of B_1- and B_2-receptors (Regoli & Barabé, 1980; Bhoola *et al*., 1992). The activation of B_1-receptors evokes contraction in rabbit (Whalley *et al*., 1983) and dog (Yen & Lai, 1992) basilar arteries, whereas the activation of B_2-receptors induces relaxation in rabbit basilar artery (Whalley *et al*., 1983). Endothelial cells play an important role in responsiveness to BK in the basilar arteries of some species. The relaxant response to BK in rats (Mayhan, 1990; Nishimura & Suzuki, 1997) and humans (Whalley *et al*., 1987) depends on the endothelium, whereas the contractile response to BK in rats (Nishimura & Suzuki, 1997) and dogs (Tsuji & Cook, 1995) does not. It is well known that BK can release different vasorelaxant factors including endothelium-derived nitric oxide (NO), cyclo-oxygenase metabolites and the so called endothelium-dependent hyperpolarizing factor (EDHF) in a different vascular bed. The EDHF component of BK response appears to play a major role in resistance arteries especially from human coronary and omental arteries (Kemp & Cocks, 1997; Ohlmann *et al*., 1997). However, it has been reported that the main relaxing factor produced by BK may be NO in rat (Mayhan, 1990), dog (Katusic *et al*., 1989) and human (Onoue *et al*., 1994) basilar arteries, and that the main contracting factor produced by BK is thromboxane (TX) A_2 in dog basilar arteries (Tsuji & Cook, 1995).

It has been reported that BK induces endothelium-dependent relaxation of porcine basilar artery (Shimokawa *et al*., 1988; Kim *et al*., 1992; Miyamoto *et al*., 1994), however, there is no information concerning the BK receptor subtype that evokes the relaxation. We recently observed that BK induced contraction after the relaxation in porcine basilar artery. The aim of the present study was to characterize the subtypes of BK receptors that evoke the relaxation and contraction in porcine basilar artery, to establish whether endothelium-dependent mechanisms are involved in this response, and to identify the main contracting and relaxing factors.

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Methods

Tissue preparation

Basilar arteries were obtained from freshly slaughtered pigs (both sexes, about 6–7 months old, LWD crossbreed) at a local slaughterhouse and transferred to our laboratory in ice-cold physiological salt solution (in mM: NaCl 119, KCl 4.7, $CaCl_2$ 1.6, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 10 (pH 7.4) aerated with carbogen (95% O_2, 5% CO_2). Each artery was dissected free of adhering tissues.

Measurement of mechanical activity

Several (2–4) rings approximately 4 mm long were cut from each artery. The rings were mounted vertically between two L-shaped stainless steel holders, which fixed the upper region to an isometric force transducer (TB-611T, Nihon Kohden Kogyo, Tokyo, Japan), in a 5-ml water-jacketed organ bath containing oxygenated salt solution at 37°C (pH 7.4). Each suspended ring was left to equilibrate for at least 120 min under a resting tension of 7.5 mN. This tension was chosen because it allowed us to induce maximal contractions in this artery. KCl (60 mM) was applied every 30 min until the amplitude of the contraction reached a constant value. Change in the KCl concentration of the physiological salt solution was compensated for by an equimolar adjustment of the NaCl concentration. The isometric tension was displayed on an ink-writing recorder (WI-641G, Nihon Kohden Kogyo, Tokyo, Japan). Cumulative concentration-response curves to BK and des-Arg^9-BK were obtained by adding a solution of the required agonist directly to the fluid in the bath. Antagonists or inhibitors were added to the bathing media 30 min prior the pre-contraction of the vessels with uridine 5′-triphosphate (UTP). There were no effects of antagonists and inhibitors on the UTP-induced contraction. The concentration of UTP chosen produced stable contractions corresponding to approximately 40% of the maximal response elicited by 60 mM KCl (10.5 ± 1.2 mN). When a competitive antagonist was tested, four rings from the same basilar artery were used to avoid tachyphylaxis which might be produced by repeating application of BK on the same preparation. To rule out the effects of BK and B_2-receptor antagonist degradation, the experiments were performed in the presence of 10^{-5} M captopril (an angiotensin converting enzyme inhibitor) and 10^{-5} M thiorphan (a neutral endopeptidase inhibitor). The log concentration-ratio of the EC_{50} values in the absence or the presence of the antagonist was calculated and plotted against the logarithm of the antagonist concentration to determine the pA_2 value. At the end of the relaxant response, papaverine (10^{-4} M) was applied to produce maximum relaxation, which was taken as 100%.

The endothelium was removed by gentle rubbing of the intimal surface with a stainless steel rod with a diameter equivalent to the lumen of the artery before the ring was mounted in the bath. The absence of endothelial cells was confirmed physiologically by testing the relaxant response to sodium fluoride ($10^{-2}-3\times10^{-2}$ M), which is abolished by endothelial denudation (Miyamoto *et al.*, 1994), and morphologically by scanning and transmission electron microscopy after the experiments.

Radioimmunoassay

Arteries for radioimmunoassay were prepared according to the method of De Caterina *et al.* (1985). Briefly, the isolated porcine basilar arteries were cut longitudinally and were rinsed several times in physiological salt solution. Each rinsed artery was incubated in physiological salt solution for 30 min at 37°C. The incubation solution was used for measurement of TXB_2, a stable metabolite of TXA_2, using a commercial kit (TXB_2 [^{3}H]-RIA KIT, New England Nuclear, Boston, U.S.A.). According to the information of this kit, the cross-reactivity of the antiserum with arachidonic acid metabolites is as follows: TXB_2, 100%; prostaglandin (PG) D_2, 0.3%; PGE_2, 0.04%; $PGF_{2\alpha}$, 0.03%; 6-keto-$PGF_{1\alpha}$, lower than 0.5%; PGA_2, lower than 0.5%. The lower limit of this kit is 2 pg TXB_2.

Drugs

Indomethacin, papaverine hydrochloride and sodium fluoride were from Nacalai (Kyoto, Japan), BK, des-Arg^9-BK, des-Arg^9,[Leu^8]-BK, nordihydroguaiaretic acid (NDGA), [Thi^{5,8}, D-Phe^7]-BK, uridine 5′-triphosphate (UTP), captopril and DL-thiorphan were from Sigma (MO, U.S.A.) and L-nitro-arginine (L-NA) was from Aldrich (Milwaukee, U.S.A.). Sodium (E)-3[4-(*l*-imidazoly-methyl)phenyl]2-propenoate (OKY-046) and the L-arginine salt of 9,11-dimethylmethano-11,12-methano-13,14- dihydro-13-aza-14- oxo-15- (β)- cyclopentyl-ω-pentanor-TXA_2 (ONO-3708) were gifts from Ono Pharmaceutical Company (Osaka, Japan). Bosentan was a gift from Hoechst Pharmaceutical Company (Frankfurt, Germany).

Statistics

Results are expressed as the mean ± s.e.mean. Statistical analyses were performed by the Bonferroni test after one-way analysis of variance. Significance was established when the probability level was equal to or less than 5%.

Results

Responsiveness to BK and des-Arg^9-BK

Typical responses to BK in isolated porcine basilar arterial rings under the resting tension and under the precontracted condition with UTP (10^{-5} M) are illustrated in Figure 1. Under resting tension, 10^{-9} and 10^{-8} M BK induced transient relaxation and 10^{-7} M BK induced relaxation followed by contraction (Figure 1A). Under the precontracted condition with UTP, BK ($10^{-9}-10^{-7}$ M) induced concentration-dependent relaxation followed by contraction (Figure 1B). Endothelial denudation completely abolished the BK-induced relaxation and contraction (Figure 1C). Therefore, the following experiments were carried out using endothelium-intact arteries. Des-Arg^9-BK ($10^{-10}-10^{-6}$ M), a B_1-receptor agonist (Regoli & Barabé, 1980), produced no response in the artery precontracted with UTP (Figure 1D).

Effects of B_1- and B_2-receptor antagonists on BK-induced relaxation and contraction

The effects of des-Arg^9,[Leu^8]-BK, a B_1-receptor antagonist (Regoli & Barabé, 1980), and [Thi^{5,8}, D-Phe^7]-BK, a B_2-receptor antagonist (Vavrek & Stewart, 1985), on BK-induced relaxation of porcine basilar arteries precontracted with UTP (10^{-5} M) are illustrated in Figure 2 and summarized in Figures 3 and 4. The difference between the UTP-induced contraction and the papaverine (10^{-4} M)-induced relaxation was taken as 100% (Figure 2A). The

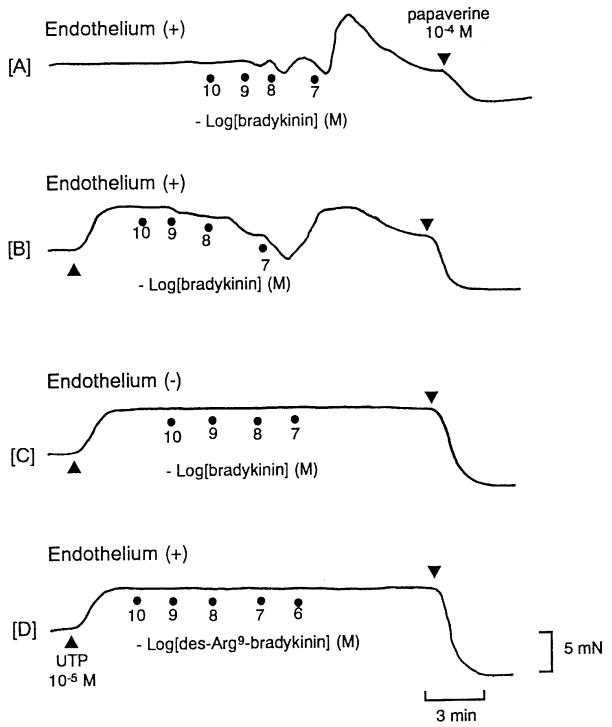

Figure 1 Typical responses to bradykinin in basilar arterial rings with endothelium under the optimal resting tension (A) and under the precontracted condition with 10^{-5} M uridine 5′-triphosphate (UTP) (B). Endothelial denudation completely abolished the bradykinin-induced responses (C). Des-Arg^9-bradykinin (a B_1-receptor agonist) had no effects on isolated porcine basilar arterial ring with intact endothelium precontracted with 10^{-5} M UTP (D).

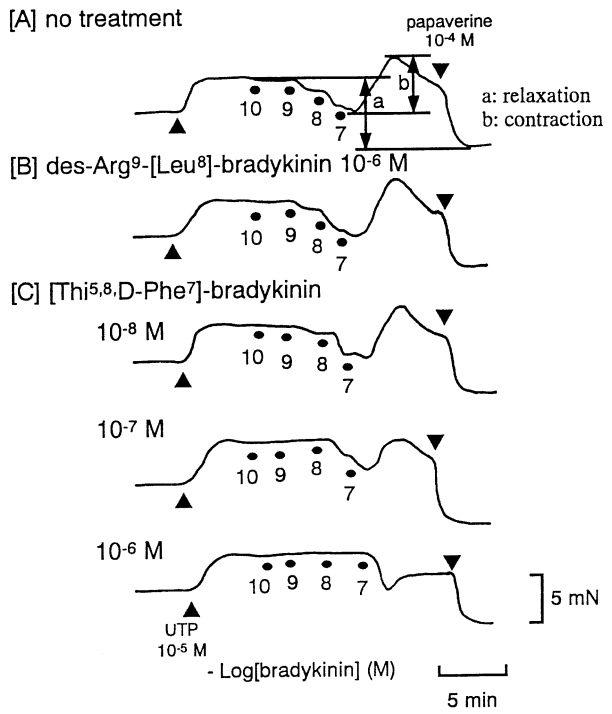

Figure 2 Typical effects of des-Arg^9, [Leu^8]-bradykinin (B) (a B_1-receptor antagonist) and [Thi^{5,8}, D-Phe^7]-bradykinin (C) (a B_2-receptor antagonist) on the bradykinin-induced relaxations (A) of endothelium-intact porcine basilar arteries precontracted with 10^{-5} M UTP. a: This difference was taken as 100% in Figure 3. b: This difference was taken as a bradykinin-induced contraction in Figures 4 and 5.

calculated pD_2 value for BK-induced relaxation was 8.48 ± 0.13 ($n=12$). [Thi^{5,8}, D-Phe^7]-BK (10^{-8}–10^{-6} M) shifted the concentration-dependent relaxant response curve of BK to the rightward, whereas des-Arg^9,[Leu^8]-BK (10^{-8}–10^{-6} M) had no significant effect on the concentration-dependent relaxant response curves of BK (Figure 3).

Figure 4 shows the effects of des-Arg^9,[Leu^8]-BK and [Thi^{5,8}, D-Phe^7]-BK on BK (10^{-7} M)-induced contraction after BK (10^{-10}–10^{-7} M)-induced relaxation in artery precontracted with UTP (10^{-5} M). The difference between the BK (10^{-7} M)-induced relaxation and the following contraction was taken as a BK-induced contraction (see Figure 2A). [Thi^{5,8}, D-Phe^7]-BK (10^{-8}–10^{-6} M) inhibited BK-induced contraction in a concentration-dependent manner, whereas des-Arg^9,[Leu^8]-BK (10^{-8}–10^{-6} M) had no significant effect on BK-induced contraction.

Effects of various inhibitors on BK-induced contraction

The effects of bosentan (a non-slective endothelin receptor antagonist) (Moreau *et al*., 1996), NDGA (a lipoxygenase inhibitor) (Yu, 1995), OKY-046 (a TXA_2 synthetase inhibitor) (Yokoyama *et al*., 1993), ONO-3708 (a TXA_2/PGH_2 receptor antagonist) (Shirahase *et al*., 1987) and indomethacin (a cyclo-oxygenase inhibitor) (Farmer *et al*., 1991) on BK (10^{-7} M)-induced contraction are shown in Figure 5. ONO-3708 (10^{-5} M) significantly inhibited BK-induced contraction, and indomethacin (10^{-5} M) completely abolished it, whereas bosentan (10^{-6} M), NDGA (10^{-5} M) and OKY-046 (10^{-4} M) had no significant effect on BK-induced contraction. These antagonists and inhibitors had no effect on BK-induced relaxation which was completely inhibited by 10^{-4} M L-NA (a NO synthase inhibitor).

In the presence of 10^{-4} M L-NA, BK induced only concentration-dependent contraction, with a pD_2 value of 7.80 ± 0.23 ($n=10$). Indomethacin (10^{-7}–10^{-5} M) inhibited BK-induced contraction in a concentration-dependent manner ($n=5$) and 10^{-5} M indomethacin completely abolished the BK-induced contraction. ONO-3708 (10^{-7}–10^{-5} M) inhibited BK-induced contraction in a concentration-dependent manner and 10^{-5} M inhibited the BK-induced contraction by 75% ($n=5$).

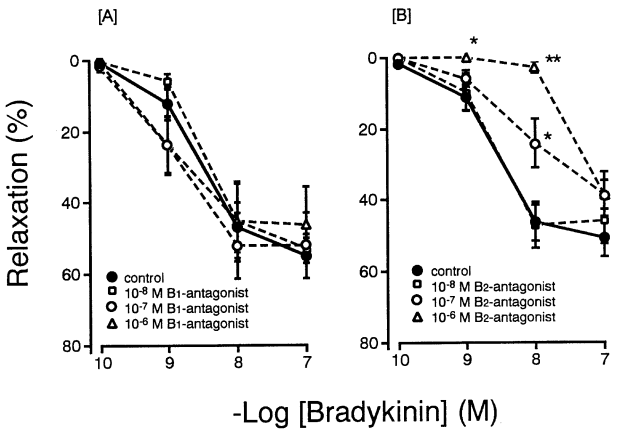

Figure 3 Effects of des-Arg^9, [Leu^8]-bradykinin (A) (a B_1-receptor antagonist) and [Thi^{5,8}, D-Phe^7]-bradykinin (B) (a B_2-receptor antagonist) on the bradykinin-induced relaxation of endothelium-intact porcine basilar arteries precontracted with 10^{-5} M UTP. Each point represents the mean ± s.e.mean of six arteries from six animals. $^{**}P<0.01$, $^{*}P<0.05$ compared with the value for bradykinin-induced relaxation in the absence of antagonist by the Bonferroni test.

Measurement of TXB_2 release from basilar artery

The amount of spontaneously released TXB_2 from porcine basilar artery with intact endothelium over a 30-min period was 43.4 ± 3.8 pg mg^{-1} wet tissue ($n=3$, duplicate). The addition of BK (10^{-6} M) had no significant effect on the amount of spontaneously released TXB_2 (40.2 ± 2.4 pg mg^{-1} wet tissue, $n=3$, duplicate).

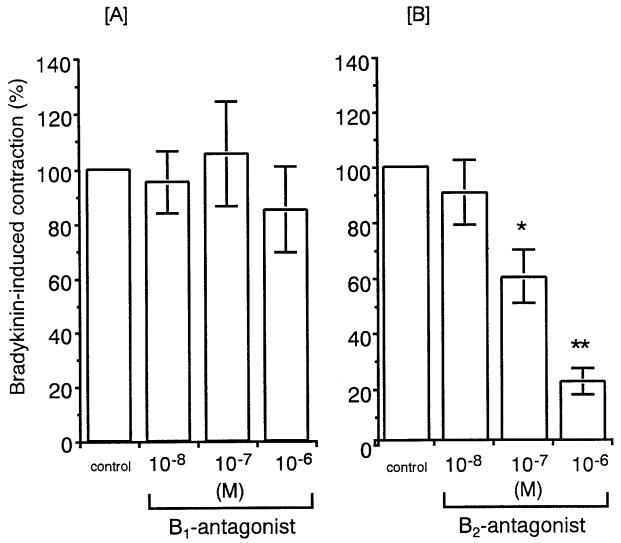

Figure 4 Effects of des-Arg^9, [Leu^8]-bradykinin (A) (a B_1-receptor antagonist) and [Thi^{5,8}, D-Phe^7]-bradykinin (B) (a B_2-receptor antagonist) on the bradykinin (10^{-7} M)-induced contraction of endothelium-intact porcine basilar arteries precontracted with 10^{-5} M UTP. Each column represents the mean$\pm$s.e.mean of seven arteries from seven animals. The value for each control was taken as 100%. $^{**}P<0.01$, $^{*}P<0.05$ compared with the value for control in the absence of antagonist by the Bonferroni test.

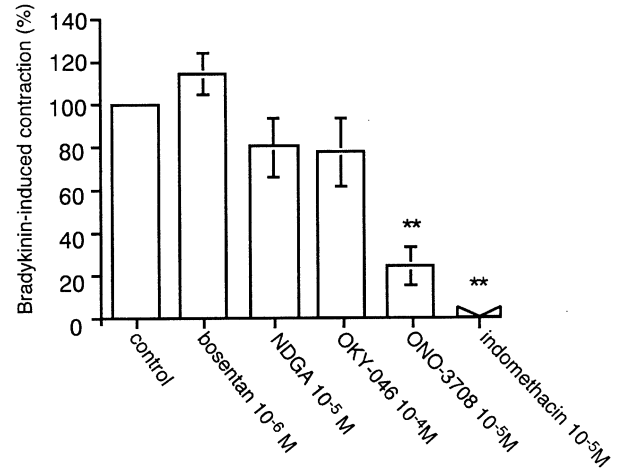

Figure 5 Effects of bosentan (a nonselective endothelin-receptor antagonist), NDGA (a lipoxygenase inhibitor), OKY-046 (a thromboxane A_2 synthetase inhibitor), ONO-3708 (a thromboxane A_2/prostaglandin H_2 receptor antagonist) and indomethacin (a cyclo-oxygenase inhibitor) on the bradykinin (10^{-7} M)-induced contraction (control) of endothelium-intact porcine basilar arteries precontracted with 10^{-5}M UTP. Each column represents the mean$\pm$s.e.mean of seven arteries from seven animals. The value for control was taken as 100%. $^{**}P<0.01$ compared with the control by the Bonferroni test.

Effect of L-NA on BK-induced relaxation in the presence of indomethacin

In the presence of 10^{-5} M indomethacin, BK induced only concentration-dependent relaxation, with a pD_2 value of 8.03 ± 0.18 ($n=6$), in artery precontracted with 10^{-5} M UTP. L-NA (10^{-6}–10^{-4} M) inhibited BK-induced relaxation in a concentration-dependent manner. 10^{-4} M L-NA completely inhibited the relaxation ($n=6$).

Comparison of pA_2 values of the B_2-receptor antagonist for BK-induced contraction and relaxation

To confirm the importance of B_2-receptors in the BK responses, B_2-receptor antagonist studies were done in the presence of indomethacin, since the contractile response was abolished, then the relaxing response to BK had occurred. On the contrary, in the presence of L-NA, the relaxing response was abolished, and the contractile response was observed. The two experiments were performed in the presence of 10^{-5} M captopril and 10^{-5} M thiorphan.

In the presence of 10^{-5} M indomethacin, [Thi^{5,8}, D-Phe^7]-BK (10^{-8}–10^{-7} M) shifted the concentration-dependent relaxant response curve of BK to the right (Figure 6). The slope value of the Schild plot for [Thi^{5,8}, D-Phe^7]-BK was 1.03 ± 0.07 ($n=8$), which was not significantly different from unity. The pA_2 value for [Thi^{5,8}, D-Phe^7]-BK was 7.59 ± 0.05 ($n=8$). In the presence of 10^{-4} M L-NA, [Thi^{5,8}, D-Phe^7]-BK (10^{-8}–10^{-7} M) shifted the concentration-dependent contractile response curve of BK to the right (Figure 7). The slope value of the Schild plot for [Thi^{5,8}, D-Phe^7]-BK was 1.02 ± 0.06 ($n=8$), which was not significantly different from unity. The pA_2 value for [Thi^{5,8}, D-Phe^7]-BK was 7.50 ± 0.06 ($n=8$). There

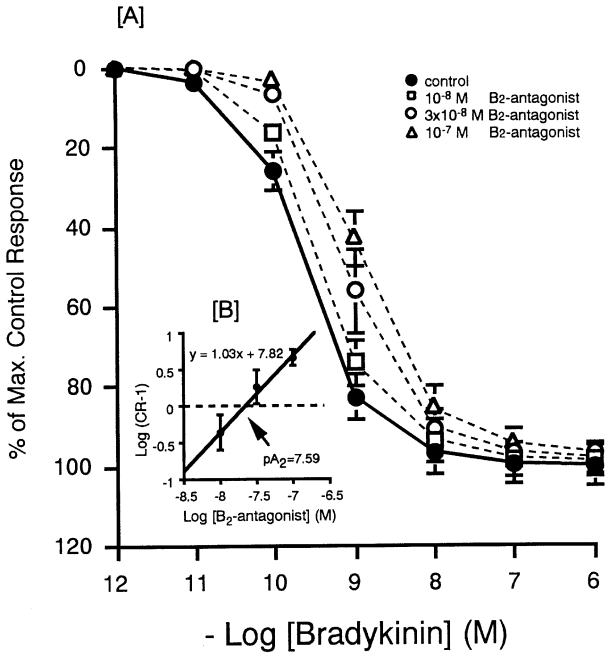

Figure 6 Effect of [Thi^{5,8}, D-Phe^7]-bradykinin (A) (a B_2-receptor antagonist) on the bradykinin-induced relaxation of endothelium-intact porcine basilar arteries precontracted with 10^{-5} M UTP in the presence of 10^{-5} M indomethacin, 10^{-5} M captopril and 10^{-5} M thiorphan. (B) Schild plot of (A). Each point represents the mean$\pm$s.e.mean of eight arteries from eight animals. Relaxation induced by 10^{-6} M bradykinin in the absence of B_2- receptor antagonist was taken as 100%.

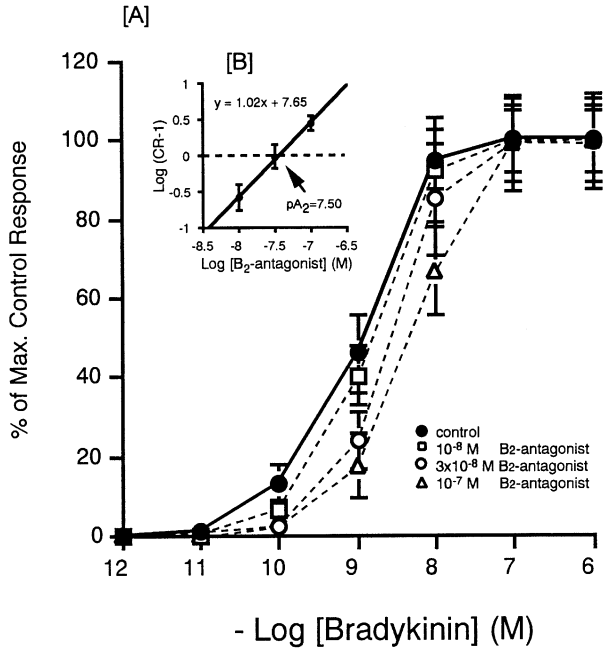

Figure 7 Effect of [Thi[5,8], D-Phe[7]]-bradykinin (A) (a B_2-receptor antagonist) on the bradykinin-induced contraction of endothelium-intact porcine basilar artery in the presence of 10^{-4} M L-NA, 10^{-5} M captopril and 10^{-5} M thiorphan. (B) Schild plot of (A). Each point represents the mean ± s.e.mean of eight arteries from eight animals. Contraction induced by 10^{-6} M bradykinin in the absence of B_2-receptor antagonist was taken as 100%.

is no significant difference of the two pA_2 values for BK-induced relaxation and contraction.

Discussion

The present study shows that both the endothelium-dependent relaxation and contraction to BK in the porcine basilar artery are mediated *via* activation of B_2-receptors on endothelial cells. The responses to BK appear to be primarily mediated *via* NO (relaxation event) and PGH_2 (contractile event), respectively.

The endothelium-dependent relaxation followed by contraction was more clearly observed in UTP-contracted artery rather than in artery at the resting tension. $PGF_{2\alpha}$ is commonly used to precontract an arterial preparation when the relaxant response is to be examined but UTP can also produce a stable contraction in basilar artery. In this study, UTP was used instead of $PGF_{2\alpha}$ to avoid the effects of exogenous PG(s). Endothelium-dependent relaxation induced by BK has been reported for rat (Nishimura & Suzuki, 1997), dog (Tsuji & Cook, 1995) and human (Whalley *et al*., 1987) basilar arteries, however, this is the first report of endothelium-dependent contraction induced by BK in basilar artery.

It is well known that endothelin (Moreau *et al*., 1996), leukotrienes (Jino *et al*., 1996), TXA_2 (Buzzard *et al*., 1993) and PGH_2 (Ito *et al*., 1991) are released from endothelial cells and act as endothelium-derived contracting factors (EDCFs). The TXA_2/PGH_2 receptor antagonist ONO-3708 strongly inhibited BK-induced contraction and the cyclo-oxygenase inhibitor indomethacin completely abolished it in this study. However, treatment with the non-selective endothelin receptor antagonist bosentan and the TXA_2 synthase inhibitor OKY-046 did not affect BK-induced contraction. BK did not increase the amount of TXB_2 release. The result supports the hypothesis that TXA_2 is not involved for BK-induced contraction in porcine basilar arteries. These results suggest that the main EDCF may be an arachidonic acid metabolite, probably PGH_2. This result was different from those reported for dog basilar artery (Tsuji & Cook, 1995) and rabbit pulmonary artery (Buzzard *et al*., 1993) in which TXA_2 was released from smooth muscle cells and endothelial cells, respectively.

BK-induced relaxation and contraction were examined separately. Porcine basilar arterial rings were pretreated with indomethacin or L-NA before adding BK. In the presence of 10^{-4} M L-NA, BK induced only contraction ($pD_2 = 7.80$). Under this condition, indomethacin and ONO-3708 inhibited BK-induced contraction in a concentration-dependent manner. On the other hand, in the presence of 10^{-5} M indomethacin, BK induced only relaxation ($pD_2 = 8.03$) in the precontracted condition with UTP. Under this condition, L-NA inhibited BK-induced relaxation in a concentration-dependent manner. These results suggest that the main relaxing factor may be endothelium-derived NO, which has previously been identified as a BK-induced relaxing factor in rat (Mayhan, 1990) and dog basilar arteries (Kinoshita & Katusic, 1997; Mombouli, 1997). It has been reported that transmural nerve stimulation causes relaxations *via* NO release in porcine basilar artery (Lee & Sarwinski, 1991), however, this response was not involved in endothelium. Therefore BK-induced relaxation did not appear to be directly concerned with a neural component. The difference of two pD_2 values of BK for the relaxation (8.03) and contraction (7.80) was small, but a little higher affinity for relaxation may be a cause of earlier appearance of relaxation to BK.

In rabbit (Whalley *et al*., 1983) and dog (Yen & Lai, 1992) basilar arteries, it has been reported that the activation of B_1-receptors evokes contraction. However, a B_1-receptor agonist, des-Arg[9]-BK, had no effect on UTP-precontracted porcine basilar arterial rings with endothelium. Furthermore, a B_1-receptor antagonist, des-Arg[9],[Leu[8]]-BK, had no effect on the concentration-dependent relaxant response to BK or on BK-induced contraction. These results suggest that activation of B_1-receptors might not be involved for BK-induced endothelium-dependent relaxation and contraction in porcine basilar arteries. A B_2-receptor antagonist, [Thi[5,8], D-Phe[7]]-BK shifted the BK-induced relaxation concentration-curves to the rightward and inhibited the BK-induced contraction concentration-dependently. These responses were abolished by endothelial denudation. These results suggest that B_2-receptors are present on porcine basilar arterial endothelial cells, and that their stimulation results in relaxation and contraction. Relaxation induced by activation of endothelial B_2-receptors has been reported in human (Whalley *et al*., 1987) and rabbit (Whalley *et al*., 1983) basilar arteries and in many other regional arteries (Bhoola *et al*., 1992), however, contraction induced by activation of endothelial B_2-receptors in basilar artery has not been reported previously. Basilar artery contraction in rabbits (Whalley *et al*., 1983) and dogs (Yen & Lai, 1992) has been reported to be mediated *via* activation of B_1-receptors on smooth muscle cells. In some veins B_2-receptor-mediated contractions (Marsault *et al*., 1997; Campos & Calixto, 1994) have been reported.

It has been reported that the coexistence of two different BK receptor subtypes (B_1 and B_2) in the same artery cause a biphasic response to BK (Persson & Andersson, 1998; Whalley *et al*., 1983). However, in the present study we showed that the only one kind of BK receptor (B_2) caused a biphasic response. The two pA_2 values for a B_2-receptor antagonist that inhibited both the BK-induced relaxation (7.59) and contraction (7.50)

did not significantly differ. In general when receptor subtypes are evaluated, it is necessary to consider the affinity and intrinsic activity in tests using agonists, whereas in test using competitive antagonists, it is enough to consider only the affinity (Gilman *et al*., 1980). In this observation, there was no significant difference in the two pA_2 values for the B_2-receptor antagonist. These results suggest that no further B_2-receptor subtypes exist in this artery. The present results rule out the possibility that BK and B_2-receptor antagonists may cause a different degradation pattern during the two experimental conditions for pA_2 determination because two experiments were performed under different inhibitory conditions of cyclo-oxygenase and nitric oxide synthase activities, respectively. The different conditions may influence cyclic AMP and cyclic GMP levels. It has been reported that cyclic AMP affect angiotensin converting enzyme and neutral endopeptidase in endothelial cells (Iwai *et al*., 1987; Dasarathy & Fanburg, 1991; Graf *et al*., 1995). It is of interest to determine how the signal from the B_2-receptor regulates the pathways of both the cyclo-oxygenase and NO-synthase systems in endothelial cells and why relaxant response was first evoked before contractile response. It has been reported that stimulation of B_2-receptors activates the NO synthase pathway (Katusic *et al*., 1989) and the cyclo-oxygenase pathway (Campos & Calixto, 1994) *via* activation of heterotrimeric G-proteins of the G_i and G_q family (Liao & Homcy, 1993; Ricupero *et al*., 1993; Miyamoto *et al*., 1997). Further studies are needed on this point.

In conclusion, BK-induced relaxation and contraction were mediated *via* activation of B_2-receptors on porcine basilar arterial endothelial cells. The main EDCF may be an arachidonic acid metabolite, probably PGH_2, whereas the main relaxing factor may be endothelium-derived NO. However, the present results do not rule out the possibility that BK-induced contraction may be mediated, at least in part, by other prostaglandins interacting with TXA_2 receptors, as observed in the rat aorta (Rapoport & Williams, 1996).

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References

BUZZARD, C.J., PFISTER, S.L. & CAMPBELL, W.B. (1993). Endothelium-dependent contractions in rabbit pulmonary artery are mediated by thromboxane A_2. *Circ. Res.,* **72,** 1023–1034.

BHOOLA, K.D., FIGUEROA, C.D. & WORTHY, K. (1992). Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol. Rev.,* **44,** 1–80.

CAMPOS, A.H. & CALIXTO, J.B. (1994). Mechanisms involved in the contractile responses of kinins in rat portal vein rings: mediation by B_1 and B_2 receptors. *J. Pharmacol. Exp. Ther.,* **268,** 902–909.

DASARATHY, Y. & FANBURG, B.L. (1991). Involvement of second messenger systems in stimulation of angiotensin converting enzyme of bovine endothelial cells. *J. Cell. Physiol.,* **148,** 327–335.

DE CATERINA, R., DORSO, C.R., TACK-GOLDMAN, K. & WEKSLER, B.B. (1985). Nitrates and endothelial prostacyclin production: studies in vitro. *Circulation,* **71,** 176–182.

EDVINSSON, L., ADAMSSON, M. & JANSEN, I. (1990). Neuropeptide Y antagonistic properties of D-myo-inositol-1.2.6-triphosphate in guinea pig basilar arteries. *Neuropeptides,* **17,** 99–105.

FARMER, S.G., ENSOR, J.E. & BURCH, R.M. (1991). Evidence that cultured airway smooth muscle cells contain bradykinin B_2 and B_3 receptors. *Am. J. Respir. Cell Mol. Biol.,* **4,** 273–277.

GILMAN, A.G., MAYER, S.E. & MELMON, K.L. (1980). Pharmacodynamics: mechanisms of drug action and the relationship between drug concentration and effect, In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, (eds). Gilman, A.G., Goodman, L.S. & Gilman, A. pp. 28–39. Macmillan Publishing Co: New York.

GRAF, K., KUNKEL, K., ZHANG, M., GRÄFE, M., SCHULTZ, K., SCHUDT, C., BIROC, S., FLECK, E. & KUNKEL, G. (1995). Activation of adenylate cyclase and phosphodiesterase inhibition enhance neutral endopeptidase activity in human endothelial cells. *Peptides,* **16,** 1273–1278.

HATAKE, K., KAKISHITA, E., WAKABAYASHI, I., SAKIYAMA, N. & HISHIDA, S. (1990). Effect of aging on endothelium-dependent vascular relaxation of isolated human basilar artery to thrombin and bradykinin. *Stroke,* **21,** 1039–1043.

ITO, T., KATO, T., IWAMA, Y., MURAMATSU, M., SHIMIZU, K., ASANO, H., OKUMURA, K., HASHIMOTO, H. & SATAKE, T. (1991). Prostaglandin H_2 as an endothelium-derived contracting factor and its interaction with endothelium-derived nitric oxide. *J. Hypertens.,* **9,** 729–736.

IWAI, N., MATSUNAGA, M., KITA, T., TEI, M. & KAWAI, C. (1987). Regulation of angiotensin converting enzyme activity in cultured human vascular endothelial cells. *Biochem. Biophys. Res. Commun.,* **149,** 1179–1185.

JINO, H., KURAHASHI, K., USUI, H., NAKATA, Y. & SHIMIZU, Y. (1996). Possible involvement of endothelial leukotrienes in acetylcholine-induced contraction in rabbit coronary artery. *Life Sci.,* **59,** 961–967.

KATUSIC, Z.S., MARSHALL, J.J., KONTOS, H.A. & VANHOUTTE, P.M. (1989). Similar responsiveness of smooth muscle of the canine basilar artery to EDRF and nitric oxide. *Am. J. Physiol.,* **257,** H1235–H1239.

KEMP, B.K. & COCKS, T.M. (1997). Evidence that mechanisms dependent and independent of nitric oxide mediate endothelium-dependent relaxation to bradykinin in human small resistance-like coronary arteries. *Br. J. Pharmacol.,* **120,** 757–762.

KIM, P., SHIMOKAWA, H. & VANHOUTTE, P.M. (1992). Dietary ω-3 fatty acids and endothelium-dependent responses in porcine cerebral arteries. *Stroke,* **23,** 407–413.

KINOSHITA, H. & KATUSIC, Z.S. (1997). Nitric oxide and effects of cationic polypeptides in canine cerebral arteries. *J. Cereb. Blood Flow Metab.,* **17,** 470–480.

LEE, T.J.-F. & SARWINSKI, S.J. (1991). Nitric oxidergic neurogenic vasodilation in the porcine basilar artery. *Blood Vessels,* **28,** 407–412.

LIAO, J.K. & HOMCY, C.J. (1993). The G proteins of the $G\alpha_i$ and $G\alpha_q$ family couple the bradykinin receptor to the release of endothelium-derived relaxing factor. *J. Clin. Invest.,* **92,** 2168–2172.

MARSAULT, R., ILLIANO, S. & VANHOUTTE, P.M. (1997). Bradykinin-induced contractions of canine saphenous veins: mediation by B_2 receptors and involvement of eicosanoids. *Br. J. Pharmacol.,* **120,** 215–220.

MAYHAN, W.G. (1990). Impairment of endothelium-dependent dilatation of basilar artery during chronic hypertension. *Am. J. Physiol.,* **259,** H1455–H1462.

MIYAMOTO, A., LAUFS, U., PARDO, C. & LIAO, J.K. (1997). Modulation of bradykinin receptor ligand binding affinity and its coupled G-proteins by nitric oxide. *J. Biol. Chem.,* **272,** 19601–19608.

MIYAMOTO, A., MATSUMOTO, M. & NISHIO, A. (1994). Endothelial modulation of vascular tone in isolated porcine and bovine basilar arteries. *J. Vet. Med. Sci.,* **56,** 947–950.

MOMBOULI, J.V. (1997). ACE inhibition, endothelial function and coronary artery lesions: role of kinins and nitric oxide. *Drugs,* **54** (suppl. 5), 12–22.

MOREAU, P., TAKASE, H. & LÜSCHER, T.F. (1996). Effect of endothelin antagonists on the responses to prostanoid endothelium-derived contracting factor. *Br. J. Pharmacol.,* **118,** 1429–1432.

NISHIMURA, Y. & SUZUKI, A. (1997). Relaxant effects of vasodilator peptides on isolated basilar arteries from stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.,* **24,** 157–161.

OHLMANN, P., MARTÍNEZ, M.C., SCHNEIDER, F., STOCLET, J.C. & ANDRIANTSITOHAINA, R. (1997). Characterization of endothelium-derived relaxing factors released by bradykinin in human resistance arteries. *Br. J. Pharmacol.,* **121,** 657–664.

ONOUE, H., KAITO, N., TOMII, M., TOKUDOME, S., NAKAJIMA, M. & ABE, T. (1994). Human basilar and middle cerebral arteries exhibit endothelium-dependent responses to peptides. *Am. J. Physiol.,* **267,** H880–H886.

PERSSON, K. & ANDERSSON, R.G.G. (1998). Biphasic response to bradykinin in isolated porcine iliac arteries is mediated by bradykinin B_1 and B_2 receptors. *J. Cardiovasc. Pharmacol.,* **31,** 306–313.

RAPOPORT, R.M. & WILLIAMS, S.P. (1996). Role of prostaglandins in acetylcholine-induced contraction of aorta from spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension,* **28,** 64–75.

REGOLI, D. & BARABÉ, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.,* **32,** 1–46.

RICUPERO, D., TAYLOR, L. & POLGAR, P. (1993). Interactions of bradykinin, calcium, G-protein and protein kinase in the activation of phospholipase A_2 in bovine pulmonary artery endothelial cells. *Agents Actions,* **40,** 110–118.

SHIMOKAWA, H., KIM, P. & VANHOUTTE, P.M. (1988). Endothelium-dependent relaxation to aggregating platelets in isolated basilar arteries of control and hypercholesterolemic pigs. *Circ. Res.,* **63,** 604–612.

SHIRAHASE, H., USUI, H., KURAHASHI, K., FUJIWARA, M. & FUKUI, K. (1987). Possible role of endothelial thromboxane A_2 in the resting tone and contractile responses to acetylcholine and arachidonic acid in canine cerebral arteries. *J. Cardiovasc. Pharmacol.,* **10,** 517–522.

TSUJI, T. & COOK, D.A. (1995). Vasoconstrictor mechanism of neuropeptides augmented after endothelial removal in isolated, perfused canine basilar arteries. *Neurol. Res.,* **17,** 193–200.

VAVREK, R.J. & STEWART, J.M. (1985). Competitive antagonists of bradykinin. *Peptides,* **6,** 161–164.

WHALLEY, E.T., AMURE, Y.O. & LYE, R.H. (1987). Analysis of the mechanism of action of bradykinin on human basilar artery in vitro. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **335,** 433–437.

WHALLEY, E.T., FRITZ, H. & GEIGER, R. (1983). Kinin receptors and angiotensin converting enzyme in rabbits basilar arteries. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **324,** 296–301.

WHALLEY, E.T. & WAHL, M. (1983). Analysis of bradykinin receptor mediating relaxation of cat cerebral arteries in vivo and in vitro. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **323,** 66–71.

YEN, M.H. & LAI, C.S. (1992). Mechanism of contractile responses to bradykinin in canine basilar arteries. *Eur. J. Pharmacol.,* **221,** 261–266.

YOKOYAMA, C., MIYATA, A., SUZUKI, K., NISHIKAWA, Y., YOSHIMOTO, T., YAMAMOTO, S., NÜSING, R., ULLRICH, V. & TANABE, T. (1993). Expression of human thromboxane synthase using a baculovirus system. *FEBS Lett.,* **318,** 91–94.

YU, S.P. (1995). Roles of arachidonic acid, lipoxygenases and phosphatases in calcium-dependent modulation of M-current in bullfrog sympathetic neurons. *J. Physiol.,* **487,** 797–811.

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