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SPECIAL REPORT

Endothelium-dependent relaxation to the B_1 kinin receptor agonist des-Arg^9-bradykinin in human coronary arteries

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Des-Arg^9-bradykinin (des-Arg^9-BK) caused endothelium-dependent relaxations in human, isolated coronary arteries which upregulated with *in vitro* incubation time. Relaxations to des-Arg^9-BK were inhibited by the B_1 receptor antagonist, des-Arg^9-[Leu^8]-BK (pK_B, 6.14±0.11) but were unaffected by the B_2 receptor antagonist, Hoe-140. Therefore, this is the first demonstration that human coronary arteries possess endothelial B_1 receptors which mediate endothelium-dependent relaxation and appear to be synthesized *de novo* during the incubation period.

Keywords: Kinin B_1 receptors; des-Arg^9-bradykinin; endothelium; human coronary artery

Introduction Bradykinin (BK) causes endothelium-dependent relaxation in isolated coronary arteries from a number of species including man (Stork & Cocks, 1994b). There is, however, only one demonstration of endothelium-dependent relaxations to des-Arg^9-BK, that in bovine coronary arteries (Drummond & Cocks, 1995). The present study is the first to show that human isolated coronary arteries also possess endothelial kinin B_1 receptors which, as in the cow (Drummond & Cocks, 1995), appear to be inducible and mediate endothelium-dependent relaxation.

Methods Ring segments (3 mm) of human left anterior descending coronary artery were suspended in 37°C Krebs solution (time zero) to record isometric force (Stork & Cocks, 1994a). In some rings of artery, the endothelium was removed by abrasion of the luminal surface with a Krebs-moistened, tapered wooden stick. Each artery ring was stretched twice to 5 g passive force, maximally contracted ($KPSS_{max}$) with 125 mM KCl (isotonic) Krebs solution and then washed with Krebs solution containing nifedipine (0.1 μM) to control phasic contractile activity (Stork & Cocks, 1994a). Each artery ring was contracted to 40% $KPSS_{max}$ with the thromboxane A_2 mimetic, U46619 (1-10 nM; Drummond & Cocks, 1995). After 3 h incubation, cumulative (0.5 log M increments) concentration-dependent relaxation curves to des-Arg^9-BK were obtained. Substance P, a recognized endothelium-dependent relaxing agonist of human coronary arteries (Stork & Cocks, 1994b) was then added to confirm the presence of the endothelium. After repeated washes with Krebs containing nifedipine the tissues were again contracted with U46619 to 40% $KPSS_{max}$, and at 6 h *in vitro* incubation, again relaxed with des-Arg^9-BK and substance P and washed. This procedure was repeated for 9 h incubation, except after the 6 h washout tissues were either untreated or treated with des-Arg^9-[Leu^8]-BK (10 μM) or Hoe-140 (0.1 μM).

Concentration-relaxation responses, normalised as percentages of the level of active force to U46619, were computer-fitted with a sigmoidal regression curve (Drummond & Cocks, 1995). Differences in mean pEC_{50} and maximum response (R_{max}) between any two groups were tested for significance by two-tailed paired *t* tests. Differences between more than two groups were analysed by repeated measures analysis of variance (ANOVA) with multiple comparisons via Tukey Kramer's modified *t* statistic. All differences were accepted as significant at the $P<0.05$ level.

Drugs Des-Arg^9-bradykinin triacetate, des-Arg^9-[Leu^8]-bradykinin triacetate, substance P triacetate (Sigma, MO, U.S.A.), U46619 (1,5,5-hydroxy-11,9-(epoxymethano) prosta-5Z,13E-dienoic acid; Upjohn, Kalamazoo, U.S.A.), (−)-nifedipine (Bayer, Australia), sodium nitroprusside (D.B.L., Australia) and Hoe-140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin; Hoechst, Australia).

Results With endothelium-intact rings of artery, 17 of 22 tested relaxed to des-Arg^9-BK, whereas all rings relaxed maximally to substance P (3 nM). After 3 h incubation R_{max} and pEC_{50} to des-Arg^9-BK were 21.7±7.1% and 6.30±0.09, respectively ($n=10$ rings from 4 patients; Figure 1). Further incubation for both 6 h and 9 h in the same rings of artery significantly ($P<0.05$) increased R_{max} to des-Arg^9-BK to 55.8±7.6% and 57.9±7.6%, respectively, without affecting the sensitivity (Figure 1). In 9 rings of artery from 3 patients, mechanical removal of the endothelium abolished relaxations to both des-Arg^9-BK (Figure 1b) and substance P (data not shown) at all incubation times. The endothelium-independent relaxing agent, sodium nitroprusside (10 μM), however, relaxed these endothelium-denuded rings maximally (data not shown).

The B_1 kinin receptor antagonist, des-Arg^9-[Leu^8]-BK (10 μM), caused an approximate 20 fold decrease in sensitivity (pK_B estimate of 6.14±0.11; $n=3$ rings from 3 patients), but did not affect the R_{max} to des-Arg^9-BK (Figure 2a). By contrast, the B_2 antagonist, Hoe 140 (0.1 μM), had no effect on relaxations to des-Arg^9-BK ($n=4$ rings from 4 patients; Figure 2b).

Discussion B_1 receptors have previously been identified on endothelial cells in culture (Sung *et al.*, 1988) and on rabbit isolated carotid (Pruneau & Belichard, 1993) and mesenteric (Churchill & Ward, 1986) and cow coronary (Drummond & Cocks, 1995) arteries. Here we show for the first time endothelium-dependent relaxations to des-Arg^9-BK in a human, isolated blood vessel, namely the left anterior descending coronary artery.

A characteristic feature of vascular responses to des-Arg^9-BK is their up-regulation under inflammatory or traumatic conditions such as *in vitro* incubation (Marceau & Regoli,

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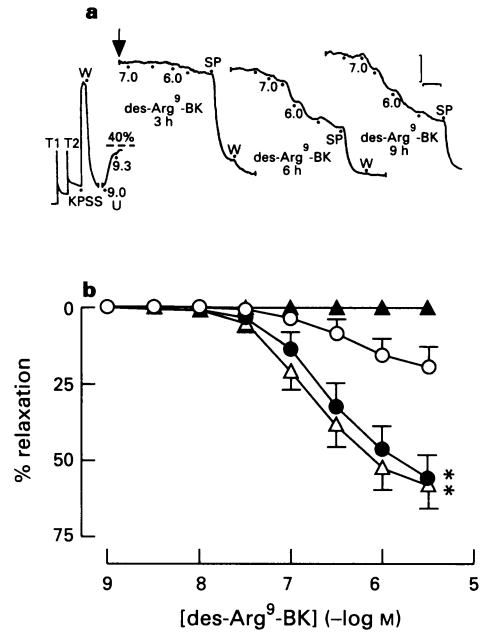

Figure 1 Relaxations to des-Arg^9-bradykinin (des-Arg^9-BK) in human isolated left anterior descending coronary artery. (a) Original chart recordings from a single artery showing sequential, time-dependent relaxations to cumulative additions of des-Arg^9-BK. T_1 and T_2; initial 5 g stretches. KPSS; maximum contraction ($KPSS_{max}$) to 125 mM KCl Krebs. SP; substance P. W; wash. The artery was contracted to 40% $KPSS_{max}$ (dotted line) with U46619 (U). Note the gain change when the contraction to U46619 had reached a stable plateau. For the 6 h and 9 h responses, the artery was similarly contracted with U46619 to approximately 40% $KPSS_{max}$ within the breaks in the trace (for clarity not shown). All concentrations are given as −log molar. The horizontal time calibration bar represents 120 min and 2 min and the vertical force calibration bar 2.5 g and 1 g, before and after the arrow, respectively. (b) Group data showing mean relaxation curves to des-Arg^9-BK at 3 h (○), 6 h (●) and 9 h (△) of incubation ($n=10$ endothelium-intact rings from 4 patients). (▲) Depicts the lack of any relaxation response to des-Arg^9-BK in endothelium-denuded artery rings ($n=9$ rings from 3 patients) at 3 h, 6 h and 9 h incubation times. Vertical lines represent s.e.mean. *Indicates maximum relaxations significantly different from those at 3 h ($P<0.05$).

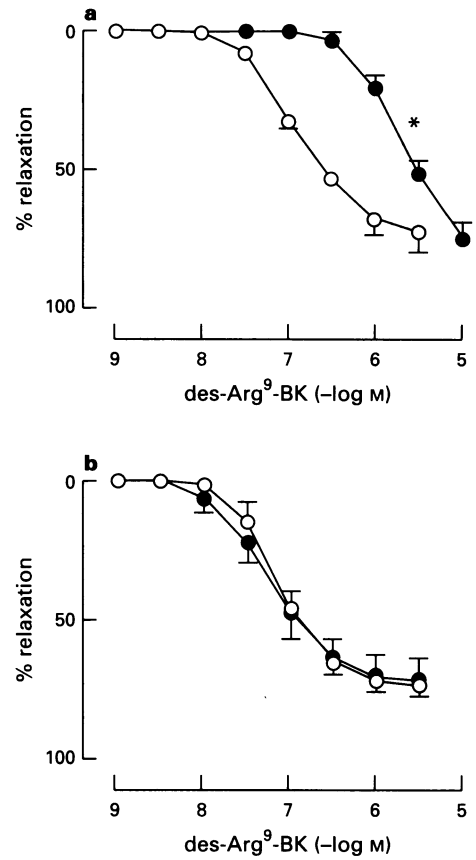

Figure 2 Effects of (a) des-Arg^9-[Leu^8]-BK (10 μM; $n=3$ rings from 3 patients) and (b) Hoe-140 (0.1 μM; $n=4$ rings from 4 patients) on the cumulative relaxation curve to des-Arg^9-BK in human isolated coronary arteries. Control responses (○) and those in the presence of both des-Arg^9-[Leu^8]-BK and Hoe 140 (●) were obtained at 6 h and 9 h incubation, respectively. *Indicates pEC_{50} values significantly different from those obtained in control tissues ($P<0.05$).

1991). Drummond & Cocks (1995) showed that upregulation of endothelium-dependent relaxation responses to des-Arg^9-BK in the cow isolated coronary artery, was inhibited by protein synthesis inhibitors and was thus probably due to the synthesis of new B_1 receptors. Whilst similar studies remain to be carried out in human coronary arteries, it is likely that relaxations to des-Arg^9-BK were mediated by B_1 receptors which upregulated with incubation time given that (1) the response to des-Arg^9-BK was antagonized by des-Arg^9-[Leu^8]-BK but not Hoe-140 and (2) the maximum relaxation response increased between 3 h and 6 h of incubation. We hypothesize that upregulation of endothelial B_1 receptors and their subsequent activation by des-Arg^9-BK *in vivo* may help to maintain coronary perfusion during disease conditions which compromise coronary blood flow, since coronary sinus kinin levels are elevated during coronary artery occlusion (Kimura *et al.*, 1973) and cardiac ischaemia is accompanied by an increase in the levels of circulating cytokines (Kamikubo, 1993), substances which can cause B_1 receptor upregulation (Galizzi *et al.*, 1995).

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