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The role of myoendothelial cell contact in non-nitric oxide-, non-prostanoid-mediated endothelium-dependent relaxation of porcine coronary artery

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1 Experiments were designed to analyse the requirement of myoendothelial junctions by bradykinin-induced endothelium-dependent relaxations resistant to N^G-nitro-L-arginine (L-NOARG) and indomethacin in porcine coronary arteries.

2 Rings of porcine coronary arteries were contracted with the thromboxane receptor agonist, U46619 and relaxations to bradykinin recorded isometrically. All experiments were performed in the presence of indomethacin. Nitric oxide (NO)-mediated effects were blocked by the NO synthase inhibitor L-NOARG (250 μM) and myoendothelial contacts inhibited by treatment with hypertonic solution containing D-mannitol or sucrose (each 180 mM) or the gap junctional uncoupling agent 1-heptanol (2 mM). High [K^+] solutions (40 mM) were used to probe a possible contribution of endothelium-derived hyperpolarizing factor (EDHF).

3 In the presence of endothelium, bradykinin induced concentration-dependent relaxations with a mean EC_{50} of 3.2 nM and a maximum response of 95 ± 1% of papaverine-induced relaxation (control curve).

4 In the absence of endothelium, bradykinin failed to induce relaxations. Addition of cultured porcine aortic endothelial cells to the organ bath resulted in some relaxation and restored in part the relaxant effect of bradykinin. This endothelial cell-mediated relaxant effect was completely abolished in the presence of 250 μM L-NOARG.

5 Bradykinin-induced relaxations in endothelium-preserved rings were only slightly suppressed by L-NOARG (86% of control). In vessels partially depolarized by high extracellular [K^+] (40 mM) relaxation was reduced to 72% of control. In the presence of L-NOARG, bradykinin failed to relax partially depolarized vessels.

6 In the presence of 2 mM 1-heptanol, 180 mM mannitol or 180 mM sucrose maximum relaxation to bradykinin was reduced to ~70%, i.e. to the same extent as in the presence of high [K^+]. The remaining relaxation was sensitive to blockade by L-NOARG.

7 Tissue cyclic GMP content which reflects NO activity, was increased about 4 fold by bradykinin (300 nM). This increase was unaffected by high [K^+], heptanol or sucrose but blocked by L-NOARG.

8 Our results suggest that non-nitric oxide- and non-prostanoid-mediated endothelium-dependent relaxation of porcine coronary artery requires functionally intact myoendothelial junctions.

Keywords: Endothelium-dependent relaxation; porcine coronary artery; myoendothelial contact; EDHF; EDRF

Introduction

Since the discovery of endothelium-dependent relaxation (Furchgott & Zawadzki, 1980) the role of endothelial cells in the regulation of vascular tone has been of great pharmacological interest. A predominant part of these endothelial functions is mediated by the endothelium-derived relaxing factor (EDRF), a labile compound that acts by a direct stimulation of the soluble guanylate cyclase in vascular smooth muscle cells. EDRF appears to be nitric oxide (NO) or a closely related compound derived from the metabolism of L-arginine by a constitutive NO synthase (Moncada *et al.*, 1991). Furthermore, evidence now exists that the endothelium releases at least one non-prostanoid relaxing factor not derived from L-arginine. For example, bradykinin has recently been shown to cause relaxation of porcine coronary artery independently of NO or guanosine 3′:5′-cyclic monophosphate (cyclic GMP) accumulation (Bény & Brunet, 1988; Richard *et al.*, 1990; Cowan & Cohen, 1991). One possible mechanism for this NO- and prostanoid-independent relaxation is membrane hyperpolarization caused by an endothelium-derived hyperpolarizing factor (EDHF) generated in various isolated vessels (Taylor & Weston, 1988; Bény & von der Weid, 1991; Vanhoutte, 1993).

Exogenous nitric oxide is without effect on membrane potential in most arteries studied, including pig coronary artery (Bény & Brunet, 1988; Komori *et al.*, 1988; Brayden, 1990). EDHF-mediated relaxation is unaffected by haemoglobin or methylene blue, which indicates that endothelium-dependent hyperpolarization is independent of the nitric oxide pathway (Chen *et al.*, 1988; Huang *et al.*, 1988). However, the exact nature of this endothelium-derived hyperpolarizing factor (EDHF) is still unknown. An important question to be answered in this respect is whether a direct communication between the endothelium and smooth muscle cells is involved, or a paracrine factor which would diffuse from the endothelium to the extracellular space.

The present study was made to evaluate the contribution of myoendothelial junctions in bradykinin-induced non-prostanoid and non-NO-mediated endothelium-dependent relaxations of porcine coronary arteries. Myoendothelial cell contact was blocked by the gap junctional uncoupling agent 1-heptanol (heptanol) or by use of hypertonic solutions containing mannitol or sucrose which cause simple cell shrinkage. To evaluate the relative contribution of endothelium-dependent hyperpolarization (presumably mediated by EDHF) and endothelium-derived NO (EDRF) to bradykinin-induced relaxations, experiments were performed in the presence of 40 mM K^+ to prevent membrane

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hyperpolarization and/or in the presence of N^G-nitro-L-arginine (L-NOARG) to inhibit generation of NO.

Methods

Tissue preparation

Porcine hearts were obtained from the local slaughterhouse and immediately transported to the laboratory in closed plastic sacs. Left and right coronary arteries were prepared without touching the intimal surface (endothelium-preserved vessels) or by gently rubbing the intimal surface with a wooden stick in order to remove the endothelium. Vessels were stored at 6°C in Krebs-Henseleit solution for up to two days.

Functional studies

Vessels were cut into rings (3–4 mm wide) which were mounted in 5 ml organ baths containing Krebs-Henseleit physiological salt solution of the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25 and glucose 10.1, maintained at 37°C, pH 7.4 and gassed with carbogen. Tension was recorded isometrically by means of F 30 force transducers connected to a Watanabe multichannel recorder (Hugo Sachs Elektronik, Freiburg, Germany). Rings were stretched to an initial tension of 2 g, and allowed to equilibrate for approximately 1 h.

All experiments were performed in the presence of indomethacin (10 μM) to block the formation of vasoactive prostanoids. Tissues were precontracted with the thromboxane receptor agonist U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (10–300 nM). Pretreatment with L-NOARG (250 μM), hypertonic solution (180 mM mannitol or sucrose), heptanol (2 mM) or KCl (40 mM) was performed 5 min (15 min for KCl) prior to precontraction. When using 40 mM K^+-containing solutions, osmolarity was adjusted by reducing Na^+ by 40 mM. In some experiments, Na^+ was kept constant (118 mM) to exclude possible effects due to Na^+ removal. Concentration-response curves for relaxing effects were obtained by cumulative addition of bradykinin (0.1–300 nM) in endothelium-preserved tissue or isoprenaline (0.1 nM–3 μM) and sodium nitroprusside (0.1 nM–10 μM) in endothelium-denuded arteries. Effects were expressed as percentages of the maximum relaxation response obtained by addition of 260 μM papaverine at the end of experiment. Basal tone was affected differently by the various agents: L-NOARG, mannitol and sucrose increased basal tone by 0.5–1.5 g (5–15% of control), and heptanol reduced basal tone by ~1.5–2 g. K^+ (40 mM) increased basal tone up to 4 g. To achieve a similar degree of precontraction, the concentration of U46619 was varied between 10–300 nM. The actual values of tone so achieved are listed in Table 1. The inhibitory effect of heptanol was only partially overcome by a maximal concentration of U46619, resulting in a ~30% lower level of precontraction, which had to be accepted.

In a second series of experiments. endothelium-denuded rings were contracted with U46619 and, once a stable tone had developed, a suspension of cultured porcine aortic endothelial cells was added to the organ bath (~3.5×10^6 cells ml^{-1}). The effects of bradykinin (100 nM) were monitored in the absence and presence of L-NOARG (250 μM). All experiments were performed in the presence of superoxide dismutase (SOD; 30 U ml^{-1}) to protect NO from inactivation by superoxide anion (Mayer *et al.*, 1993).

Cell culture

Porcine aortic endothelial cells were cultured as described previously (Graier *et al.*, 1992). Briefly, endothelial cells were isolated by 20 min of enzymatic digestion (200 U ml^{-1} collagenase), centrifuged for 5 min at 500 *g*, resuspended in Opti-Minimum Essential Medium containing 3% foetal calf serum and antibiotics and seeded in plastic Petri dishes. Confluent cells were split by a ratio of 1:5 and subcultured for up to two passages. On the day of experiment cells were harvested by treatment with 0.025% trypsin and 0.02% EDTA, centrifuged and resuspended in Krebs-Henseleit physiological salt solution at ~20×10^6 cells ml^{-1}.

Measurement of cyclic nucleotides

Cyclic GMP content was measured by radioimmunoassay in freeze clamped coronary artery rings as described earlier (Kukovetz *et al.*, 1979). Samples were taken under similar conditions as in the mechanical experiments either during control periods or after maximum stimulation with bradykinin (300 nM).

Data analysis

Concentration-response curves were analysed with the Allfit programme package (De Lean *et al.*, 1978). Fitting of individual curves yielded EC_{50} values and maximum effect values (E_{max}). EC_{50} values are expressed as geometric means with 95% confidence intervals, calculated as the product of s.e.mean × Student's *t* values, E_{max} values were calculated as arithmetic means ± s.e.mean. *n* represents the number of tissues studied. Means were compared by Student's *t* test. Differences were considered statistically significant at $P \leqslant 0.05$.

Table 1 Level of precontraction of porcine coronary artery obtained after equilibration of rings, followed by induction of tone with U46619 in the absence and presence of agents indicated

Agent (s)	*Developed tension* (g)	*U46619 used (nM)*	n
Control	10.2 ± 0.7	100	9
+ L-NOARG	11.4 ± 0.8	50–100	10
+ Heptanol	7.0 ± 1.4	300	5
+ Heptanol and L-NOARG	7.5 ± 1.1	300	5
+ Sucrose	9.8 ± 0.8	50–100	9
+ Sucrose and L-NOARG	10.5 ± 0.5	50–100	9
+ Mannitol	9.6 ± 1.1	50–100	5
+ Mannitol and L-NOARG	11.5 ± 0.9	50–100	5
+ KCl	11.2 ± 0.7	10–30	5
+ KCl and L-NOARG	11.8 ± 0.9	10–30	5

Means ± s.e.mean of *n* tissues.

Materials

Bradykinin, N^G-nitro-L-arginine, U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$), D-mannitol, collagenase (Type II), trypsin and EDTA (ethylenediaminetetracetic acid) were purchased from Sigma (Munich, Germany). Indomethacin sodium trihydrate was from Merck, Sharp & Dohme (Munich, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany), and tissue culture media from sources described previously (Graier *et al.*, 1992).

Results

Endothelium-preserved rings of porcine coronary artery contracted with the thromboxane receptor agonist, U46619 were potently relaxed by bradykinin (0.1–300 nM) with an EC_{50} value of 3.2 (1.8–5.6) nM (geometric mean with 95% confidence interval) and a maximum response of 95 ± 1% (arithmetic mean ± s.e.mean; $n = 9$). Pretreatment of rings with the NO synthase inhibitor, L-NOARG (250 μM), shifted the concentration-response curve to the right ($EC_{50} = 11.5$ (5.2–25.6) nM and diminished E_{max} to 86% of controls ($n = 10$) (Figure 1).

In endothelium-free preparations no relaxation to bradykinin was observed. Addition of cultured porcine aortic endothelial cells in the presence of SOD (30 U ml^{-1}) resulted in some relaxation and partly restored the relaxant effect to bradykinin (100 nM, $n = 4$). This endothelium-mediated relaxant effect was completely blocked by 250 μM L-NOARG ($n = 4$). A representative experiment is shown in Figure 2.

In vessels depolarized by high extracellular $[K^+]$ (40 mM), the bradykinin relaxation curve was shifted even further to the right than in the presence of L-NOARG ($EC_{50} = 28.8$ (9.2–90.0) nM) and E_{max} was reduced to 72% of control ($n = 5$). In the combined presence of L-NOARG and high $[K^+]$, however, relaxations to bradykinin were almost completely abolished (8% of control, $n = 5$) (Figure 3). The experiments of this group were carried out in isotonic (low Na^+) solutions (see Methods). When Na^+ was kept constant resulting in a slightly (20%) hypertonic solution, similar results were obtained ($n = 3$, data not shown).

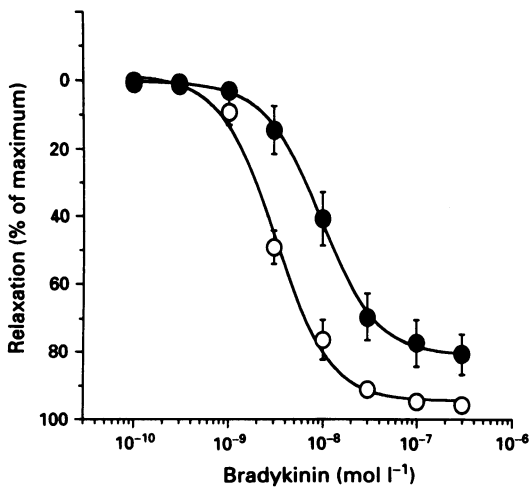

Figure 1 Bradykinin-induced relaxant effects in U46619-contracted rings of endothelium-preserved porcine coronary artery in the absence (control curve: ○) or presence of 250 μM N^G-nitro-L-arginine (●). Results are expressed as percentage of the maximum relaxation obtained by addition of 260 μM papaverine at the end of the experiment. Data points represent the mean ± s.e.mean of 9–10 individual experiments.

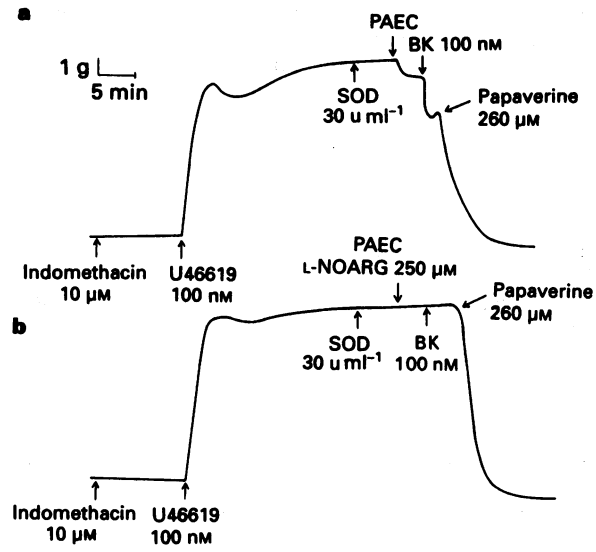

Figure 2 Effects of addition of porcine aortic endothelial cells (PAEC) to coronary artery rings without endothelium. (a) Rings were precontracted with U46619, and ~10×10^6 PAECs (~3.5×10^6 cells ml^{-1} final concentration) were then added to the organ bath, followed by bradykinin (BK, 100 nM) and, finally, papaverine (260 μM). (b) Same experimental set-up, but in the presence of 250 μM N^G-nitro-L-arginine (L-NOARG). Superoxide dismutase (SOD, 30 U ml^{-1}) was added to protect NO from inactivation.

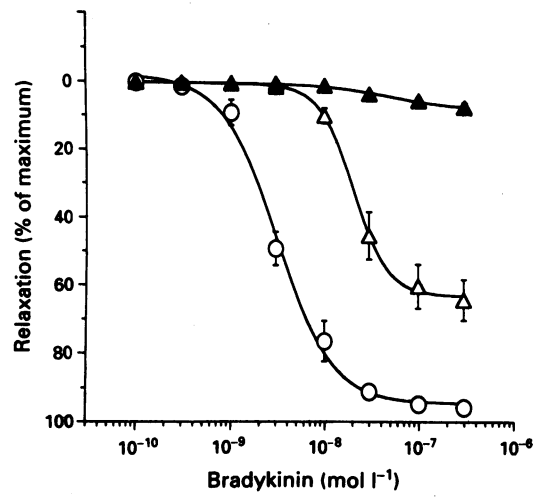

Figure 3 Bradykinin-induced relaxant effects in U46619-contracted rings of endothelium-intact porcine coronary artery in the absence (control curve: ○) and presence of 40 mM KCl (△) or 40 mM KCl plus 250 μM N^G-nitro-L-arginine (▲). Results are expressed as percentage of the maximum relaxation obtained by addition of 260 μM papaverine at the end of the experiment. Data points represent the mean ± s.e.mean of 5 individual experiments.

In the presence of the gap junctional uncoupling agent, heptanol (2 mM), E_{max} was reduced to 71%, i.e. to the same extent as in the presence of high $[K^+]$, and the EC_{50} was not significantly affected ($EC_{50} = 4.2$ (1.7–10.3) nM, $n = 5$). The remaining relaxation was sensitive to blockade by L-NOARG resulting in a suppression of E_{max} to 27% of control in the combined presence of heptanol and L-NOARG ($n = 5$) (Figure 4).

In vessels treated with hypertonic solution containing either 180 mM mannitol (Figure 5) or sucrose (Figure 6), relaxations to bradykinin were shifted to the right with an EC_{50} of 12.0 (4.1–34.9) nM (mannitol) and 19.5 (10.3–36.9) nM (sucrose). Maximum relaxations were reduced to 70%

(mannitol, $n = 5$) and 73% (sucrose, $n = 9$) of control, i.e. to the same extent as in the presence of high [K^+]. The remaining relaxations were again sensitive to blockade by L-NOARG. In the combined presence of hypertonic solution and L-NOARG, relaxations were reduced to 48% of control (mannitol, $n = 5$) and 47% (sucrose, $n = 9$), respectively.

Endothelium-independent relaxations to isoprenaline were unaffected by heptanol, sucrose, or mannitol. Control EC_{50} values were 60.3 (28.4–128) nM in the absence of inhibitors, and in their presence 66.1 (37–118) nM (heptanol), 54 (23.0–128) nM (mannitol), and 39.0 (13.5–81.5) nM (sucrose), respectively. E_{max} values ranged from 96–99% of controls ($n = 6$, data not shown). Similar results were obtained with sodium nitroprusside ($n = 7$, data not shown).

The effects of L-NOARG, heptanol, sucrose and K^+ on tissue cyclic GMP content of porcine coronary arteries are summarized in Table 2. Basal cyclic GMP content was 34.0 ± 4.9 pmol g^{-1} wet weight under control conditions, and increased to 129.8 ± 18.8 pmol g^{-1} wet weight (3.8 fold, $n = 10$) at maximum effective concentrations of bradykinin. A similar increase was seen in the presence of a depolarizing concentration of K^+, heptanol or sucrose ($n = 5$), whereas in the presence of L-NOARG or in the combined presence of L-NOARG plus heptanol, sucrose or K^+, both basal and stimulated cyclic GMP levels were reduced ($n = 5$) (Table 2).

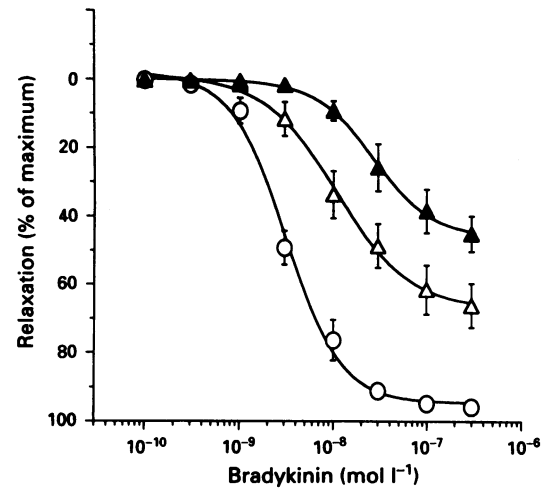

Figure 5 Bradykinin-induced relaxant effects in U46619-contracted rings of endothelium-intact porcine coronary artery in the absence (control curve: O) and presence of 180 mM mannitol (Δ) and 180 mM mannitol plus 250 μM N^G-nitro-L-arginine (▲). Results are expressed as percentage of the maximum relaxation obtained by addition of 260 μM papaverine at the end of the experiment. Data points represent the mean ± s.e.mean of 5 individual experiments.

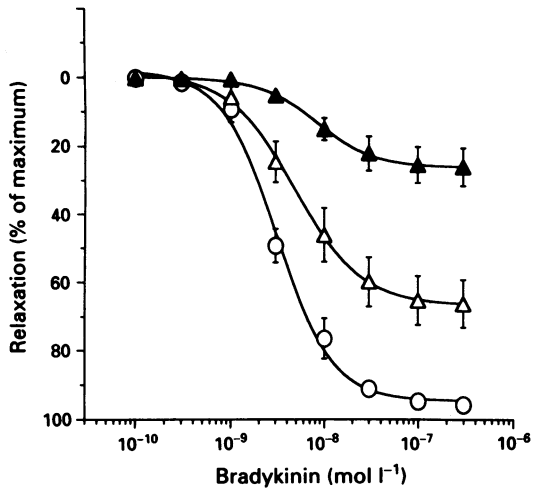

Figure 4 Bradykinin-induced relaxant effects in U46619-contracted rings of endothelium-preserved porcine coronary artery in the absence (control curve: O) and presence of 2 mM heptanol (Δ) or 2 mM heptanol plus 250 μM N^G-nitro-L-arginine (▲). Results are expressed as percentage of the maximum relaxation obtained by addition of 260 μM papaverine at the end of the experiment. Data points represent the mean ± s.e.mean of 5 individual experiments.

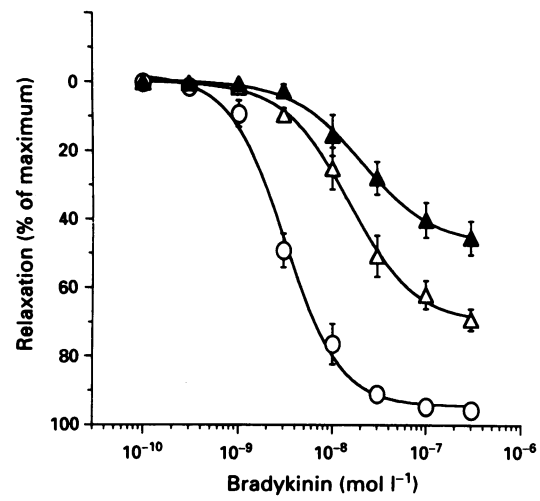

Figure 6 Bradykinin-induced relaxant effects in U46619-contracted rings of endothelium-intact porcine coronary artery in the absence (control curve: O) and presence of 180 mM sucrose (Δ) or 180 mM sucrose plus 250 μM N^G-nitro-L-arginine (▲). Results are expressed as percentage of the maximum relaxation obtained by addition of 260 μM papaverine at the end of the experiment. Data points represent the mean ± s.e.mean of 9 individual experiments.

Table 2 Cyclic GMP levels (pmol g^{-1} wet weight) during basal conditions and after stimulation with bradykinin (300 nM)

	Basal	*Bradykinin*	*-fold increase over basal*
Control	34.0 ± 4.9	129.8 ± 18.8	3.8
+ L-NOARG (250 μM)	9.6 ± 0.9	11.6 ± 1.5	
+ Heptanol (2 mM)	25.9 ± 4.2	137.5 ± 22.6	5.3
+ Heptanol (2 mM) and L-NOARG (250 μM)	10.2 ± 1.7	13.7 ± 4.3	
+ Sucrose (180 mM)	28.7 ± 4.7	110.1 ± 19.5	3.8
+ Sucrose (180 mM) and L-NOARG (250 μM)	8.4 ± 1.9	10.3 ± 1.5	
+ KCl (40 mM)	34.4 ± 9.2	99.3 ± 21.3	2.9
+ KCl (40 mM) and L-NOARG (250 μM)	7.5 ± 0.9	8.4 ± 1.3	

Cyclic GMP content was measured by radioimmunoassay in freeze clamped coronary artery rings as described in Methods. Samples were taken under similar conditions as in the mechanical experiments either during control periods (basal condition) or after stimulation with 300 nM bradykinin when maximum relaxation had developed. Values are given as arithmetic means ± s.e.mean of 5–10 individual experiments.

Discussion

The present study demonstrates that in porcine isolated coronary arteries bradykinin elicits endothelium-dependent relaxations that, (i) are not mediated by NO or prostacyclin, (ii) require close myoendothelial contact, and (iii) are inhibited to the same extent by either blockade of myoendothelial junctions or membrane depolarization with high [K^+].

A number of studies demonstrate that in porcine coronary arteries, bradykinin induces endothelium-dependent relaxations insensitive to blockade by L-NOARG and indomethacin (Kauser & Rubanyi, 1992; Nagao & Vanhoutte, 1992; Pacicca *et al.*, 1992). This observation shows that in porcine coronary arteries an endothelium-derived relaxing factor distinct from NO or prostacyclin may mediate the relaxation to bradykinin and thereby contribute to the local regulation of vascular tone. In the present study, experiments were designed to determine whether this L-NOARG-insensitive component is mediated by a diffusible factor and/or requires the existence of an intact junctional communication between endothelium and vascular smooth muscle cells.

Coronary artery rings with endothelium intact showed L-NOARG-sensitive relaxation which amounted to ~15% of the maximum inducible by bradykinin. In the absence of endothelium, no relaxation was observed which is in agreement with published data. Addition of endothelial cells to arteries without endothelium, however, resulted in a relaxation which was entirely inhibited by L-NOARG. The extent of this relaxation was similar to that mediated by NO in intact artery rings (~15%). Hence, the larger part of the total relaxation observed was mediated by a mechanism independent of NO- and prostanoids which apparently require cell-to-cell-contact. Simple addition of cultured endothelial cells, however, did not restore such NO- and/or prostanoid-independent relaxation in endothelium-denuded arteries. This may be explained if the active mediator of this relaxant component is either extremely labile and is ineffective in the present experimental set-up or if this relaxation requires tight electrical coupling between endothelial and vascular smooth muscle cells.

In our study we have blocked myoendothelial communication by the well-known gap junctional uncoupling agent, heptanol. Electrophysiological and optical imaging techniques have shown that heptanol as well as other long chain alcohols are able to block reversibly junctional transfer of current-carrying ions, second messenger molecules and fluorescent dyes in diverse cell types (Bennett *et al.*, 1991; Christ *et al.*, 1992). Moreover, gap junctions can be split by treating intact tissues with hypertonic solution (Loewenstein, 1981).

Our results clearly show that the gap junction inhibitors heptanol, mannitol, and sucrose are able to antagonize endothelium-dependent relaxations resistant to NO and prostanoids. This effect was rather specific since these blockers did not affect endothelium-independent relaxation induced by isoprenaline or sodium nitroprusside. The extent of blockade induced by these uncoupling agents was similar in all three cases (~30% reduction of maximum effect). Accordingly, close myoendothelial contact is paramount for the transfer of the relaxation signal from the endothelium to the smooth muscle.

It is of note that the gap junction blockers at the concentrations used did not entirely suppress non-NO relaxations. This may be due to the nature of these blockers which, although used at standard concentrations, may not entirely disrupt myoendothelial contact. Although we have no proof of this at present, we consider this as the probable cause for the remaining relaxation observed. Failing this, an additional hypothetical relaxant component may have to be postulated.

The nature of the physical contact between endothelial and smooth muscle layer necessary for bradykinin-induced relaxation is presently unknown. Cytoplasmatic bridges passing through fenestrations of internal elastic lamina connecting endothelium to smooth muscle cells of arteries of various calibres have been detected which could be involved in vasorelaxation, either independent of or in concert with endothelial humoral factors such as NO (Davies *et al.*, 1988). According to this hypothesis, the two tissues would function as a coupled system allowing smooth muscle responses to secondary signals generated in the endothelium and transferred through such junctions. However, it is still unclear whether the endothelial and smooth muscle cells in arteries and small arterioles are electrically coupled via gap junctions because dyes fail to diffuse via myoendothelial junctions between these cells *in situ* (Bény, 1990), although coupling between smooth muscle cells and endothelial cells was observed in co-culture (Davies *et al.*, 1985).

Another suggestion as to how endothelium-dependent agonists might mediate vasorelaxation resistant to NO and prostanoids concerns a paracrine hyperpolarizing factor (EDHF) (Feletou & Vanhoutte, 1988; Kauser *et al.*, 1989; Chen *et al.*, 1991). Consistent with this proposal, hyperpolarization of the smooth muscle layer has been observed repeatedly (Bolton *et al.*, 1984; Komori & Suzuki, 1987; Bény & Brunet, 1988; Chen *et al.*, 1988; Feletou & Vanhoutte, 1988; Nagao & Vanhoutte, 1992). To determine if hyperpolarization might be involved in this junctional transfer of the relaxant signal, we used high extracellular [K^+] which permits the differentiation between endothelium-dependent relaxations mediated by NO (EDRF) and hyperpolarization (EDHF) (Nagao & Vanhoutte, 1992; Adeagbo & Triggle, 1993). Most interestingly, use of high extracellular [K^+] resulted in a similar inhibition (i.e., to 70% of control) of bradykinin-induced relaxation as blockade of myoendothelial junctions by gap junction blockers. Thus, hyperpolarization, although not measured in this study, makes an important contribution to bradykinin-induced relaxation of porcine coronary artery as shown before (Bény & Brunet, 1988; Nagao & Vanhoutte, 1992). The present data strongly suggest that the endothelium-derived signal requires junctional cell-to-cell communication. Whether this signal is chemical (EDHF) or electrical in nature, remains to be shown.

Elevation of extracellular [K^+] above 40 mM is known to depolarize markedly smooth muscle and to antagonize the effects of various hyperpolarizing agents, including those of EDHF. We cannot exclude the possibility that part of the effects of high extracellular [K^+] is due to depolarization of endothelial cells, particularly since EDHF release is a Ca^{2+}-dependent phenomenon (Vanhoutte, 1993) and endothelial Ca^{2+} signals are depressed by K^+-depolarization (Groschner *et al.*, 1992). Such an effect is, however, unlikely, since endothelial NO-production which is also Ca^{2+}-dependent, was unaffected as evident from cyclic GMP levels measured in tissue rings (Table 2).

Endothelium-dependent relaxation of vascular smooth muscle is known to be associated with an increase in tissue cyclic GMP levels (Kukovetz *et al.*, 1979). In the present study bradykinin-stimulated accumulation of cyclic GMP was completely inhibited by L-NOARG, a specific blocker of NO production but unaffected by the blockers of myoendothelial junctions, heptanol, sucrose and mannitol, and by high [K^+]. Thus, NO and cyclic GMP are not critical for complete bradykinin-induced relaxation in porcine coronary artery, and there is at least one relaxing component which acts independently of cyclic GMP, and is blocked by depolarization of the cell membrane, or by inhibiting myoendothelial communication.

In summary, the present study demonstrates that bradykinin-induced endothelium-dependent relaxation in porcine coronary arteries requires close myoendothelial contact. This component of relaxation is independent of NO production and cGMP accumulation and is not mediated by vasoactive prostanoids. The data are compatible with EDHF or any other messenger mediating the NO-insensitive component of relaxation, providing that functional myoendothelial junctions are present.

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