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Characterization of endothelium-derived relaxing factors released by bradykinin in human resistance arteries

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1 Relaxing factors released by the endothelium and their relative contribution to the endothelium-dependent relaxation produced by bradykinin (BK) in comparison with different vasodilator agents were investigated in human omental resistance arteries.

2 BK produced an endothelium-dependent relaxation of arteries pre-contracted with the thromboxane A_2 agonist, U46619. The B_2 receptor antagonist, Hoe 140 (0.1, 1 and 10 μM), produced a parallel shift to the right of the concentration-response curve to BK with a pA_2 of 7.75.

3 Neither the cyclo-oxygenase inhibitor, indomethacin (10 μM) alone, the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME, 300 μM) alone, the nitric oxide scavenger, oxyhaemoglobin (Hb, 10 μM) alone, nor the combination of L-NAME plus Hb affected the concentration-response curve to BK. Conversely, the combination of indomethacin with either L-NAME or Hb attenuated but did not abolish the BK-induced relaxation. By contrast, the relaxations produced by the Ca^{2+} ionophore, calcimycin (A23187), and by the inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, thapsigargin (THAPS), were abolished in the presence of indomethacin plus L-NAME. Also, the presence of indomethacin plus L-NAME produced contraction of arteries with functional endothelium.

4 The indomethacin plus L-NAME resistant component of BK relaxation was abolished in physiological solution (PSS) containing 40 mM KCl and *vice versa*. However, in the presence of KCl 40 mM, indomethacin plus L-NAME did not affect the nitric oxide donor, S-N-acetylpenicillamine-induced relaxation.

5 The indomethacin plus L-NAME resistant component of the relaxation to BK was significantly attenuated by the K^+ channel blocker tetrabutylammonium (TBA, 1 mM). However, it was not affected by other K^+ channel blockers such as apamin (10 μM), 4-aminopyridine (100 μM), glibenclamide (10 μM), tetraethylammonium (10 mM) and charybdotoxin (50 nM).

6 In the presence of indomethacin plus L-NAME, the relaxation produced by BK was not affected by the phospholipase A_2 inhibitor, quinacrine (10 μM) or by the inhibitor of cytochrome P450, SKF 525a (10 μM). Another cytochrome P450 inhibitor, clotrimazole (10 μM) which also inhibits K^+ channels, inhibited the relaxation to BK.

7 These results show that BK induces endothelium-dependent relaxation in human small omental arteries via multiple mechanisms involving nitric oxide, cyclo-oxygenase derived prostanoid(s) and another factor (probably an endothelium-derived hyperpolarizing factor). They indicate that nitric oxide and cyclo-oxygenase derivative(s) can substitute for each other in producing relaxation and that the third component is not a metabolite of arachidonic acid, formed through the cytochrome P-450 pathway, in these arteries.

Keywords: Endothelium; endothelium-derived hyperpolarizing factor (EDHF); human resistance arteries

Introduction

It is well established that endothelium plays an important role in the regulation of vascular tone. Indeed, vascular endothelium can release constricting factors (Lüscher *et al*., 1992) such as endothelin, angiotensin II or endoperoxide and relaxing factors such as nitric oxide (NO) (Palmer *et al*., 1987), prostacyclin, and an endothelium-derived hyperpolarizing factor (EDHF), the nature of which remains to be determined (Chen *et al*., 1988; Taylor *et al*., 1988). Bradykinin (BK) is one of the major endogenous substances producing endothelium-dependent vasodilatation, thereby playing an important role in tissue perfusion and peripheral resistance. The aim of the present study was to investigate further the mechanisms of BK action in human resistance arteries.

In general, the release of endothelium derived factors requires an increase in intracellular calcium ($[Ca^{2+}]_i$) in the endothelial cells (Long & Stone, 1985; Peach *et al*., 1987) which is due to both Ca^{2+} influx and Ca^{2+} release from intracellular stores. However, an increase in $[Ca^{2+}]_i$ is not required for prostaglandin release from cultured endothelial cells stimulated with acetylcholine, indicating that the releasing mechanisms may differ between the relaxing factors (Lückohff *et al*., 1988). In human arteries, only a few studies have investigated the existence and the relative contribution of the relaxing factors in the endothelium-dependent relaxation produced by different agonists such as BK or substance P (Nakashima *et al*., 1993; Deng *et al*., 1995; Petersson *et al*., 1995; Wallerstedt & Bodelsson, 1997). These studies indicate that the endothelium-dependent relaxation involves mainly NO and EDHF. However, the participation of prostacyclin in this phenomenon is still unclear.

Endothelium-derived relaxing factors released by BK have been characterized, in the present study, in small omental arteries and the effects of BK were compared to those of compounds known to induce an increase in $[Ca^{2+}]_i$ in endothelial cells, i.e. the Ca^{2+} ionophore, calcimycin (A23187) and an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, thapsigargin (THAPS).

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Methods

Arterial preparation and mounting

Omental arteries were isolated from pieces of human epiploon harvested for histopathology in patients requiring a large bowel resection for cancer or inflammatory disease. The experimental procedures were performed according to the ethical guidelines of the 1989 modified Helsinki declaration. In addition, written consent was obtained from each patient. None of the patients (mean age 63 ± 16 years, sex ratio 1:1, $n = 22$) demonstrated a systemic inflammatory response syndrome or had received any cardiovascular therapy.

Omental arteries were collected into cold physiological salt solution (PSS) with the following composition (in mM): NaCl 119, KCl 4.7, KH_2PO_4 0.4, $NaHCO_3$ 14.9, $MgSO_4$ 1.17, $CaCl_2$ 2.5 and glucose 5.5. Then, the arteries were cleaned of fat and connective tissue and a segment 2 mm long was removed. The segment was then mounted in a myograph filled with PSS kept at 37°C and continuously gassed with a mixture of 95% O_2, 5% CO_2 (pH 7.4). Briefly, two tungsten wires (30 μm diameter) were inserted through the lumen. Mechanical activity was recorded isometrically by a force transducer (Kistler-Morse, DSG BE4), connected to one of the two tungsten wires, the other being attached to a support carried by a micromanipulator.

After being mounted, the vessel was equilibrated for 30 min before being passively stretched to an internal diameter that yielded a circumference equivalent to 90% of that given by an internal pressure of 100 mmHg; this required a load of about 400 mg. The internal diameter of the vessels used in this study ranged between 200–400 μm, corresponding to resistance vessels defined by Mulvany & Aalkjaer (1990).

Thirty min after the vessel had been set to its working length, it was challenged with the combination of depolarizing solution, (i.e. KCl (100 mM)-PSS) and a maximally active concentration of the thromboxane agonist U46619 (9, 11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$) to test the maximal contractile capacity of the vessel. The presence of functional endothelium was assessed in all preparations by the ability of BK (1 μM) to induce more than 60% relaxation of vessels pre-contracted to 80% of their maximal response with U46619. In some experiments, the endothelium was removed by intraluminal perfusion with 0.5% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulphonate (CHAPS) for 30 s followed by repeated washings with normal PSS. The vessels were considered denuded of their endothelium when BK (1 μM) failed to produce any relaxation of U46619 pre-contracted vessels.

Experimental protocols

Arteries with and without functional endothelium were pre-contracted to 80% of maximal contraction with U46619. The concentration of U46619 was adjusted for each preparation. When the contraction reached a plateau, cumulative addition of different vasodilator agents was performed (i.e., BK, the Ca^{2+} ionophore, A23187 and the inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, THAPS).

Two or three concentration-response curves to either BK, A23187 or THAPS were successively constructed in arteries with functional endothelium separated by washout periods of 30 min. Each artery was used with one of the above relaxing agents only. Control experiments had shown that there was no significant difference between these curves without any other treatment, and the first curve constructed in normal PSS was then taken as control.

The second curve was constructed either in normal PSS in the presence of the indicated inhibitor(s) or in 40 mM KCl-PSS without any inhibitor added. The following inhibitors were used: the B_2 receptor antagonist, Hoe 140 (0.1, 1 and 10 μM), the cyclo-oxygenase inhibitor, indomethacin (10 μM), the NO scavenger, oxyhaemoglobin (Hb, 10 μM), the NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME, 300 mM).

The third curve was established either in 40 mM KCl-PSS in the presence of indomethacin plus L-NAME or in normal PSS in the presence of indomethacin plus L-NAME combined with one of the following inhibitors: a blocker of the large conductance Ca^{2+}-activated K^+ channel (BK_{Ca}), tetraethylammonium (TEA, 10 mM), tetrabutylammonium (TBA, 1 mM) or charybdotoxin (CTX, 50 nM); a blocker of the small conductance Ca^{2+}-activated K^+ channel (SK_{Ca}), apamin (10 μM); a blocker of the ATP-activated K^+ channel (K_{ATP}), glibenclamide (10 μM); a blocker of voltage gated K^+ channel (K_v), 4-aminopyridine (100 μM); an inhibitor of phospholipase A_2 (PLA_2), quinacrine (10 μM) and a cytochrome P450 inhibitor, SKF 525a (10 μM) or clotrimazole (10 μM).

To study the relaxation induced by S-N-acetylpenicillamine (SNAP), two concentration-response curves were performed. The first curve was constructed in normal PSS without any inhibitor added, and the second curve was performed in 40 mM KCl in the presence of indomethacin plus L-NAME.

When indomethacin was used in combination with L-NAME or Hb, the concentration of U46619 was adjusted in order to obtain the same level of pre-contraction. All the inhibitors were used at a maximally active concentration and were incubated with the tissue for 15 min before the pre-contraction with U46619.

Drugs

Bradykinin, A23187, THAPS, L-NAME, Hb, sodium dithionite, indomethacin, TEA, TBA, charydobtoxin, apamin, glibenclamide, 4-aminopyridine, quinacrine, clotrimazole and CHAPS were purchased from Sigma Chemical (Grenoble, France). U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$) from Cayman Chemical Company (USA), Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]BK) from Hoechst (Paris, France) and SNAP from ICN Biomedicals Inc (USA). SKF 525a (α-phenyl-α-propylbenzeneacetic acid 2-[diethylamino] ethyl ester) was a generous gift of Dr Mansuy (CNRS URA 400, Paris, France). Stock solutions were prepared in distilled water (Q10, Millipore) except for indomethacin (dissolved in 5% $NaHCO_3$), clotrimazole and THAPS (in absolute ethanol) and glibenclamide (in dimethyl sulphoxide). Reduced Hb was prepared by treatment of the Hb solution with sodium dithionite according Martin *et al*. (1985).

Expression of results and statistical analysis

The maximal contractions obtained with U46619 (1 μM) were not significantly different being 1.48 ± 0.12 g ($n = 19$) and 1.42 ± 0.28 ($n = 8$) in vessels with or without endothelium, respectively. Results are expressed as a percentage of the relaxation from the initial U46619-induced pre-contraction level. The concentration of U46619 was adjusted for each preparation to obtained 80% of the maximal response. All results are expressed as mean ± s.e.mean of n experiments; n represents the number of patients. Sensitivities to agonists are expressed as pD_2 values, where $pD_2 = -\log EC_{50}$, EC_{50} being the molar concentration of the agonist that produces 50% of the maximal effect; EC_{50} values were calculated by logit-log regression. $pA_2 = -$logarithm of the molar concentration reducing the effect of the agonist to half of the original effect. The pA_2 values were calculated by linear regression. Analysis of variance (MANOVA) was used to compare the concentration-curves to vasodilator agents between each group of preparations. Differences were considered significant when $P < 0.05$.

Results

BK, A23187 and THAPS produced relaxations in a concentration-dependent manner in arteries with functional endothelium pre-contracted with U46619 (Figure 1). All the vasorelaxant agents failed to produce relaxation in endothelium-denuded arteries.

The B_2 receptor antagonist, Hoe 140 produced a parallel shift to the right of the concentration-response curves to BK in a concentration-dependent manner without affecting the maximal response (Figure 2). The corresponding calculated pA_2 value of Hoe 140 was 7.75 ± 0.35 (the slope value of the Schild plot was 0.82 ± 0.19 and was not significantly different from unity).

Neither the cyclo-oxygenase inhibitor, indomethacin (10 μM) alone (Figure 3a), the NO synthase inhibitor, L-NAME (300 μM) alone (Figure 3b), Hb alone (Figure 3c), nor the combination of Hb plus L-NAME (Figure 3d) affected the concentration-response curve to BK. However, the combination of indomethacin plus L-NAME (Figure 3e; $P<0.05$) or Hb (Figure 3f; $P<0.05$) partially inhibited the BK-induced relaxation (Table 1). Also, neither indomethacin alone, Hb alone nor L-NAME alone changed the basal tone of the arteries, whereas the combination of indomethacin plus L-NAME produced contraction ($29 \pm 9\%$ of maximal contraction produced by 3 μM U46619, $n=15$, not shown) of arteries with functional endothelium. Finally, indomethacin plus L-NAME abolished the relaxations produced by both A23187 and THAPS (Figure 4). Comparison of the effects of indomethacin plus L-NAME at an identical level of relaxation

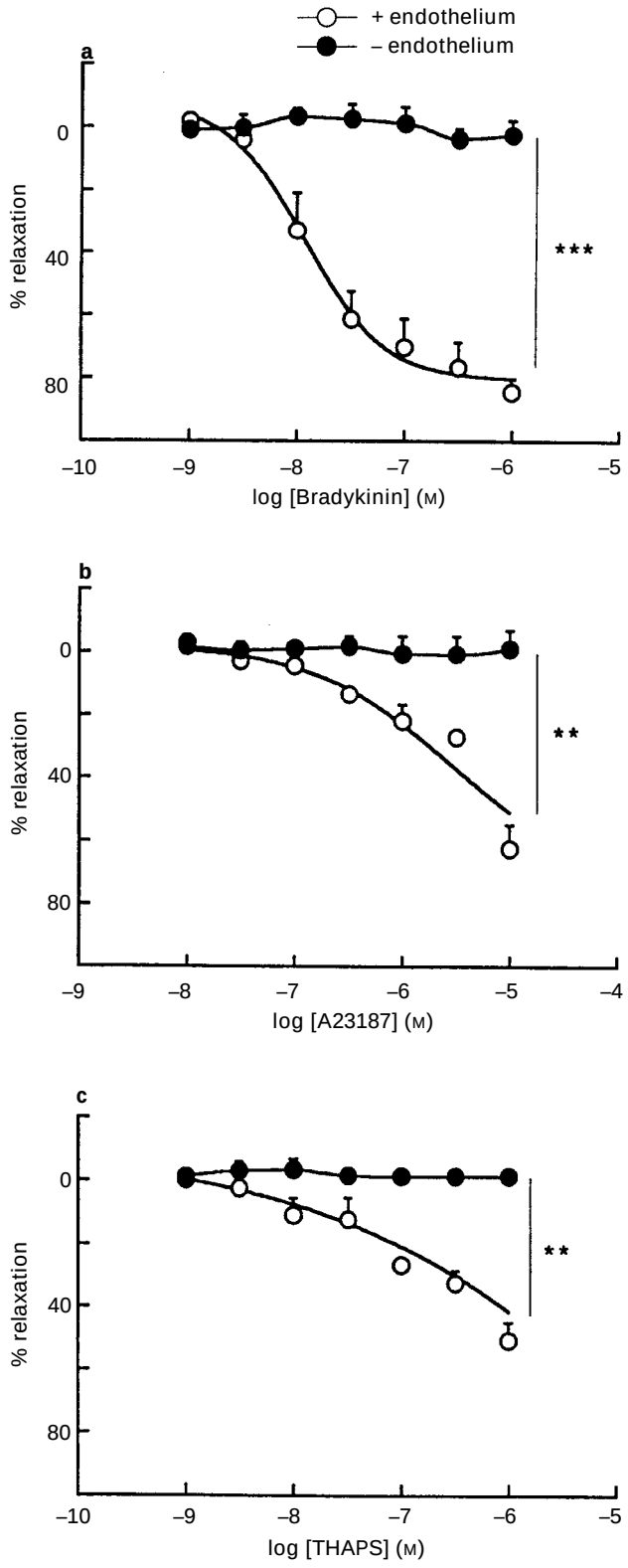

Figure 1 Concentration-response curves to bradykinin (a), A23187 (b) and thapsigargin (THAPS, c) of human omental resistance arteries pre-contracted with the thromboxane agonist U46619 in the presence (○) and absence (●) of functional endothelium. The level of pre-contraction induced by U46619 was (g) 1.46 ± 0.13 (BK), 1.44 ± 0.16 (A23187) and 1.50 ± 0.23 (THAPS). The points are mean $\pm$ s.e.mean (vertical lines) of 5–7 experiments. $^{**}P<0.01$, $^{***}P<0.001$ significantly different as compared to with endothelium.

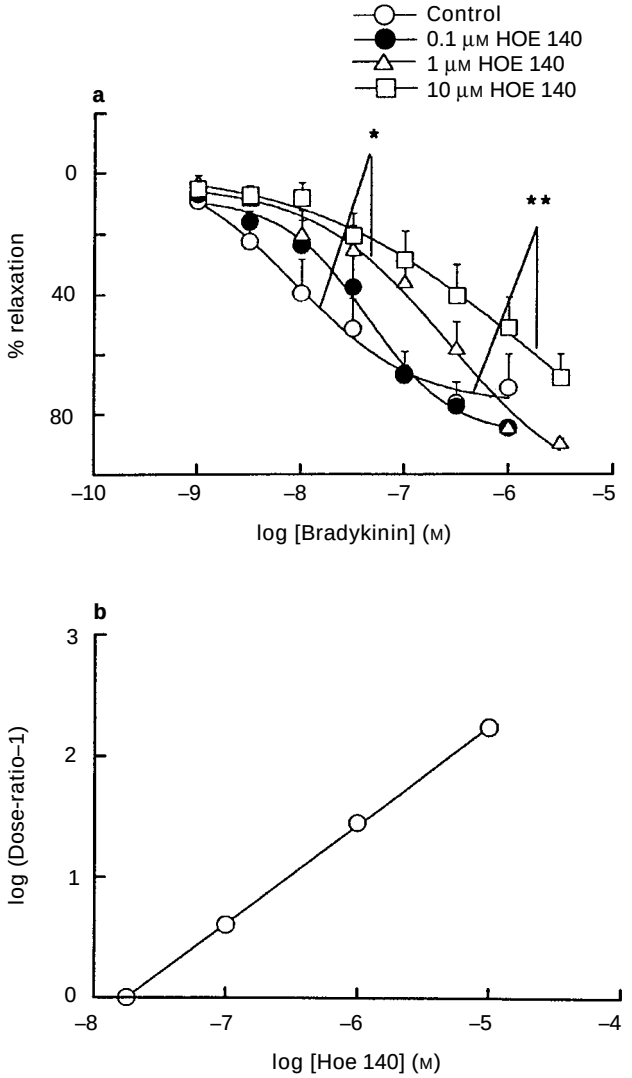

Figure 2 Concentration-response curves to bradykinin (a) and corresponding Schild plot (b) of results obtained in the absence (○) and in the presence of the B_2 receptor antagonist, Hoe 140 (0.1 μM, ●; 1 μM, △; 10 μM, □) in human omental small arteries. The level of pre-contraction induced by U46619 was (g) 1.43 ± 0.13 (control), 1.44 ± 0.55 (Hoe 0.1 μM), 1.33 ± 0.04 (Hoe 1 μM) and 1.42 ± 0.16 (Hoe 10 μM). The points are mean $\pm$ s.e.mean (vertical lines) of 5–7 experiments. $^{*}P<0.05$, $^{**}P<0.01$, significantly different as compared to controls.

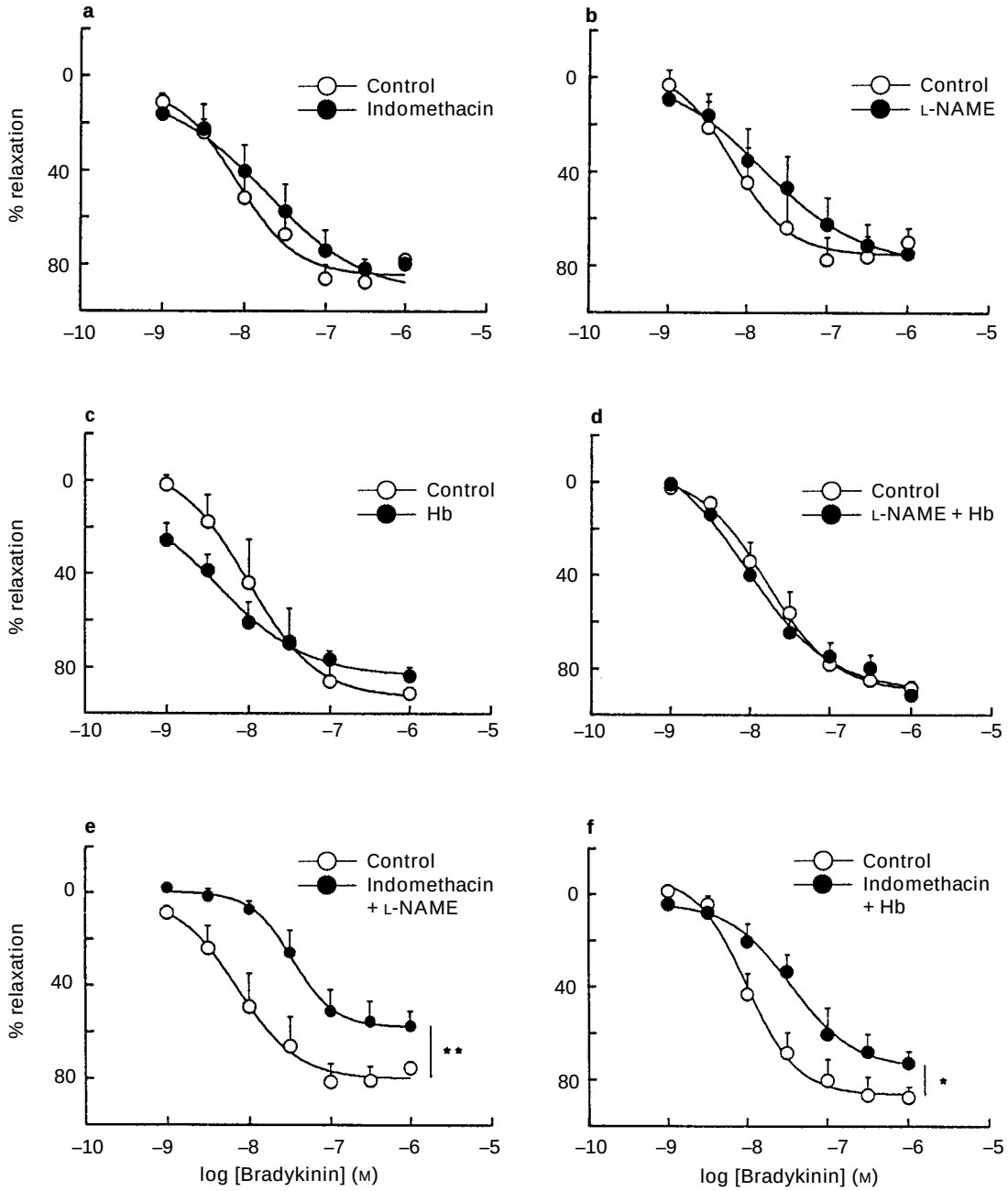

Figure 3 Concentration-response curves to bradykinin obtained in the absence (○) and in the presence (●) of (a) indomethacin (10 μM), (b) N^{ω}-nitro-L-arginine methyl ester (L-NAME, 300 μM), (c) oxyhaemoglobin (Hb, 10 μM), (d) L-NAME plus Hb, (e) indomethacin plus L-NAME or (f) indomethacin plus Hb in human small omental arteries. The level of pre-contraction induced by U46619 was (g) 1.40 ± 0.21 (control), 1.44 ± 0.24 (indomethacin), 1.41 ± 0.3 (L-NAME), 1.41 ± 0.16 (Hb), 1.38 ± 0.25 (L-NAME plus Hb), 1.38 ± 0.09 (indomethacin plus L-NAME) and 1.39 ± 0.3 (indomethacin plus Hb). The points are mean $\pm$ s.e.mean (vertical lines) of 5–7 experiments. $^{*}P<0.05$, $^{**}P<0.01$ significantly different as compared to controls.

(50%) shows that relaxation was abolished in the case of A23187 and THAPS (Figure 4), but not in the case of an equiactive concentration of BK (0.03 μM, Figure 5b). At this concentration, BK still induced a significant relaxation ($28 \pm 9\%$, $P<0.05$) in the presence of indomethacin plus L-NAME. Thus, differential inhibition of relaxation induced by BK, on the one hand, and A23187 and THAPS, on the other hand, was not solely due to a difference in the level of relaxation.

The indomethacin plus L-NAME resistant component of the vasorelaxing effect of BK was further investigated by changing the bath from normal PSS to 40 mM KCl-PSS in order to depolarize the arteries. This produced an increase in basal tone (5–10% of the maximal contraction, not shown). For relaxation experiments, the concentration of U46619 was adjusted (0.03–0.1 μM) to obtain the same level of pre-contraction as in normal PSS (i.e., 80% of the maximal contraction elicited by U46619 in normal PSS). In the absence of indomethacin and L-NAME, exposure to 40 mM KCl-PSS produced partial inhibition of BK-induced relaxation (Figure 5a) comparable to the inhibition caused by exposure to indomethacin plus L-NAME in normal PSS (Figure 5b). Further addition of indomethacin plus L-NAME in 40 mM KCl-PSS abolished the effect of BK (Figure 5a). Exposure to 40 mM KCl-PSS in the continuous presence of indomethacin plus L-NAME also abolished the effect of BK (Figure 5b). Conversely, the concentration-response curve of the NO donor S-N-acetylpenicillamine (SNAP) (0.01–1 μM) was not significantly affected by exposure to 40 mM KCl-PSS in the presence of indomethacin plus L-NAME (Figure 6).

In order to characterize the mechanism(s) involved in the indomethacin plus L-NAME-insensitive response to BK, the effects of several K^+ channel blockers were investigated. As illustrated in Figure 7, among the blockers of BK_{Ca} only TBA (Figure 7b) partially inhibited the indomethacin plus L-NAME-insensitive component of the BK-induced relaxation, TEA and charybdotoxin being inactive. All the other K^+ channel blockers tested (i.e., the blocker of the SK_{Ca}, apamin, the blocker of K_v, 4-aminopyridine and the blocker of K_{ATP}, glibenclamide) did not affect the indomethacin plus L-NAME-insensitive component of the BK-induced relaxation (Table 2).

The PLA_2 inhibitor quinacrine (10 μM) was without effect on the indomethacin plus L-NAME-insensitive response to BK (Table 2). Also, the cytochrome P450 inhibitor, SKF 525a (10 μM) did not affect the indomethacin plus L-NAME-insensitive response to BK (Table 2). Clotrimazole (10 μM), another cytochrome P450 inhibitor which also inhibits K^+ channels, slightly but significantly attenuated the indomethacin plus L-NAME-insensitive responses to high concentrations of BK (i.e. 0.3 μM and 1 μM) (Table 2).

Table 1 pD_2 values and maximal effects (E_{max}) of bradykinin (BK) in human small omental arteries pre-contracted with the thromboxane A_2 agonist, U46619

Treatment	*pD_2*	*E_{max} (% relaxation)*
Control	8.09±0.16	87.6±5.1
+indomethacin	7.74±0.22	81.9±4.3
Control	8.18±0.14	77.5±8.4
+L-NAME	7.80±0.12	74.8±7.4
Control	8.01±0.04	91.1±3.8
+Hb	8.37±0.28	86.8±6.8
Control	7.79±0.16	89.5±5.8
+Hb+L-NAME	8.04±0.21	81.5±7.8
Contol	8.14±0.12	83.7±4.3
+indomethacin+L-NAME	7.47±0.03*	57.3±6.6*
Control	8.01±0.07	84.7±5.3
+indomethacin+Hb	7.45±0.08*	72.5±7.8

Values are mean±s.e.mean of 5–7 experiments. *Significantly different ($P<0.05$) from the corresponding control values.

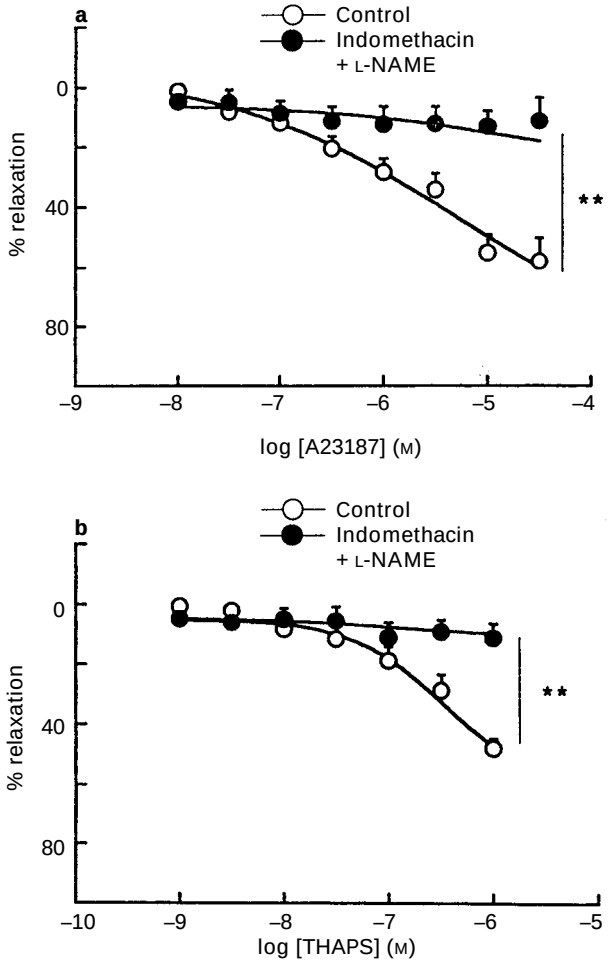

Figure 4 Concentration-response curves to A23187 (a) and to thapsigargin (THAPS, b) obtained in the absence (○) and in the presence (●) of indomethacin (10 μM) plus N^{ω}-nitro-L-arginine methyl ester (L-NAME, 300 μM) in human small omental arteries. The level of pre-contraction induced by U46619 was (g) 1.49±0.29 (A23187 control), 1.37±0.13 (A23187 plus indomethacin plus L-NAME), 1.44±0.16 (THAPS control) and 1.25±0.07 (THAPS plus indomethacin plus L-NAME). The points are mean±s.e.mean (vertical lines) of 5–7 experiments. **$P<0.01$ significantly different as compared to controls.

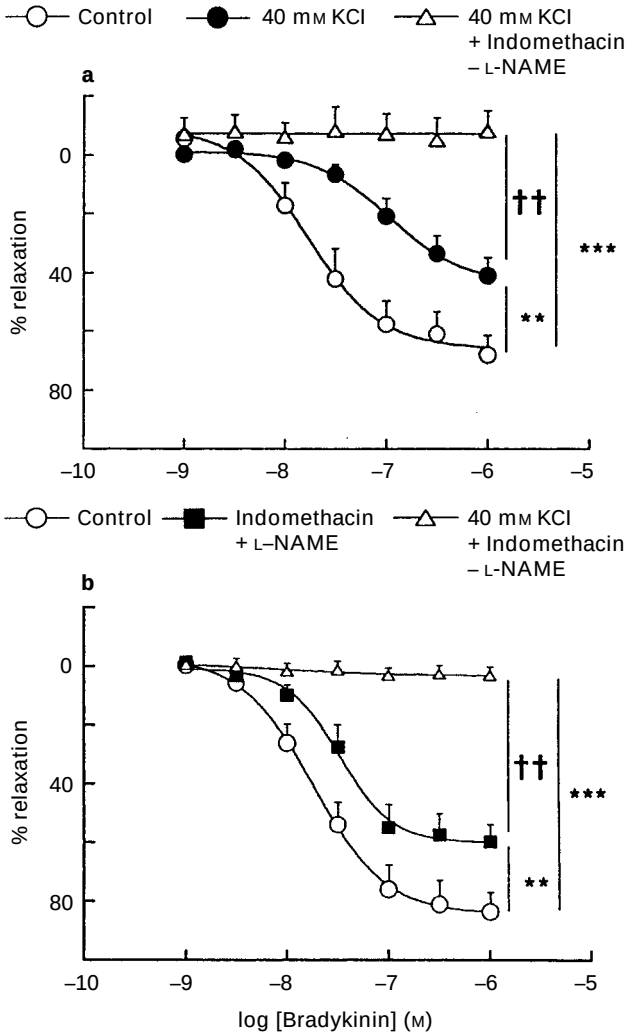

Figure 5 Concentration-response curves to bradykinin obtained in normal physiological solution (PSS) (○) and in PSS containing 40 mM KCl (●, a), in the presence of indomethacin (10 μM) plus N^{ω}-nitro-L-arginine methyl ester (L-NAME, 300 μM) in normal physiological solution (PSS) (■, b) and in PSS containing 40 mM KCl in the presence of indomethacin (10 μM) plus N^{ω}-nitro-L-arginine methyl ester (L-NAME, 300 μM) (△, a and b) in human small omental arteries. The level of pre-contraction induced by U46619 was (g) 1.32±0.21 (control, a), 1.38±0.19 (control, b), 1.29±0.12 (40 mM KCl), 1.35±0.23 (indomethacin plus L-NAME, b) and 1.36±0.22 and 1.31±0.25 (40 mM KCl plus indomethacin plus L-NAME, a and b, respectively). The points are mean±s.e.mean (vertical lines) of 5–7 experiments. **$P<0.01$, ***$P<0.001$ significantly different as compared to controls. ††$P<0.01$ significantly different as compared to the relaxation obtained in the presence of indomethacin plus L-NAME (a) or in PSS containing 40 mM KCl (b).

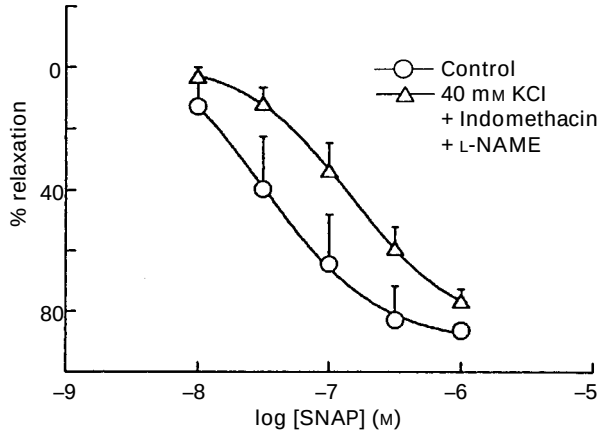

Figure 6 Concentration-response curves to S-N-acetylpenicillamine (SNAP) obtained in normal physiological solution (PSS) (○) and in PSS containing 40 mM KCl, indomethacin (10 μM) plus N^{ω}-nitro-L-arginine methyl ester (L-NAME, 300 μM) (△) in human small omental arteries. The level of pre-contraction induced by U46619 was (g): 1.36±0.12 (control) and 1.38±0.15 (40 mM KCl plus indomethacin plus L-NAME). The points are mean±s.e.mean (vertical lines) of 5–7 experiments.

Table 2 Effects of different K^+ channel blockers and phospholipase A_2 and cytochrome P-450 inhibitors on pD_2 and E_{max} of the indomethacin plus L-NAME resistant relaxation to bradykinin in human omental resistance arteries

Treatment	pD_2	E_{max} *(% relaxation)*
L-NAME + indomethacin	7.24±0.05	69.8±6.4
+ Apamin	7.20±0.01	74.9±5.5
+ 4-aminopyridine	7.32±0.06	66.1±7.7
+ Glibenclamide	7.28±0.03	71.5±8.2
+ SKF525a	7.18±0.04	69.2±5.4
+ Clotrimazole	6.99±0.04	26.9±9.9*
+ Quinacrine	7.26±0.03	63.9±6.2

Values are mean±s.e.mean of 5–7 experiments. *Significantly different ($P<0.05$) from the corresponding values of pD_2 in the presence of L-NAME plus indomethacin.

Discussion

The above results demonstrate the respective participation of endothelium-derived factors in vasorelaxation induced by BK in human omental small arteries. They show that simultaneous inhibition of both NO-synthase and cyclo-oxygenase is required to impair endothelium-dependent relaxation, suggesting that one of the two pathways is able to substitute for the other. In addition, they show the involvement of another mechanism, which is abolished in KCl (40 mM) exposed vessels and attenuated by TBA, an inhibitor of BK_{Ca}.

It has been shown that endothelial cells contain B_2 BK receptors (Keravis *et al*., 1991) the activation of which by BK induces the release of endothelial relaxing factors (Schini *et al*., 1990) and accounts for the vasorelaxant effect of BK at the concentrations used here (pD_2 around 8). However, these receptors had not been characterized previously in human small omental arteries. In the present experiments, the B_2 selective antagonist, Hoe 140, competitively displaced to higher values the concentration-effect curve of BK, consistent with the involvement of B_2-receptors. However, the pA_2 value of Hoe 140 found (7.75) appeared to be lower than values obtained previously: 8.42 in guinea-pig isolated ileum (Hock *et al*., 1991). The existence of differences in the affinity of B_2 receptor antagonists has been proposed as possibly reflecting the existence of receptor subtypes or species differences (Receptor and Ion Channel Nomenclature Supplement, 1996).

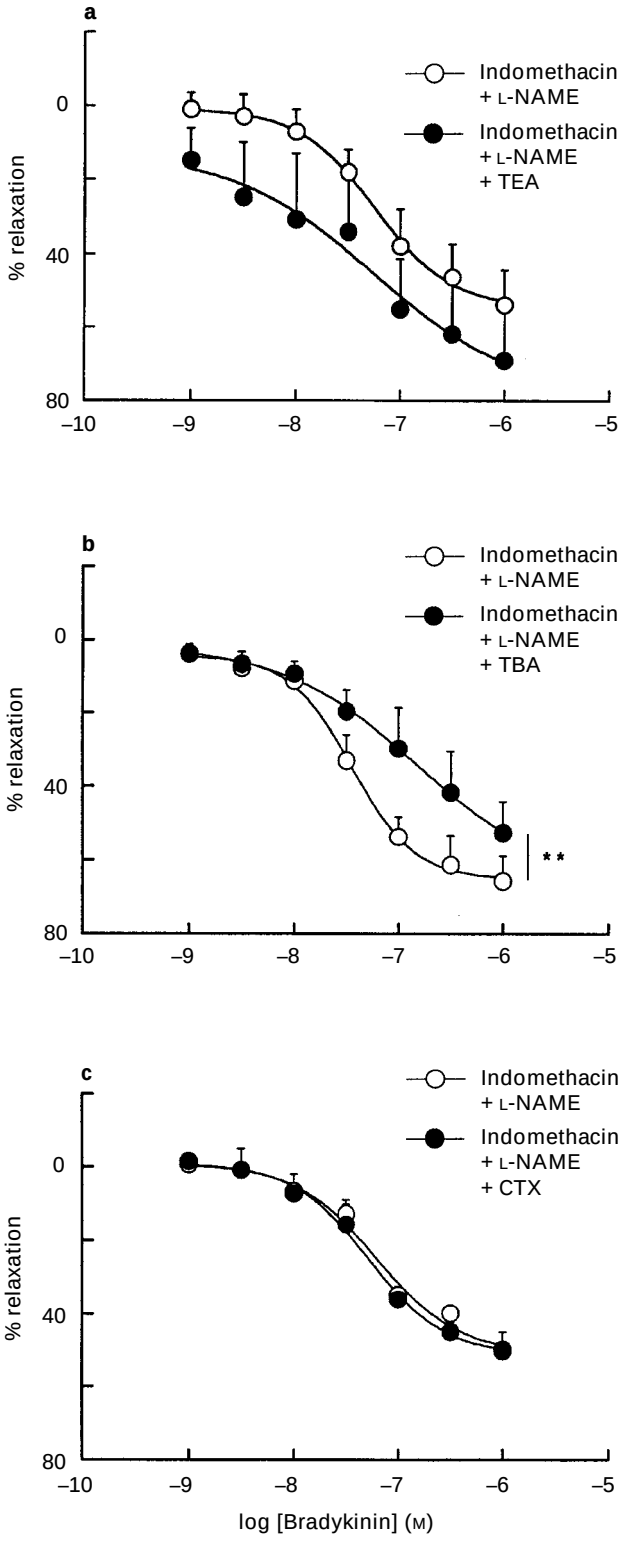

Figure 7 Concentration-response curves to bradykinin obtained in the presence of indomethacin (10 μM) plus N^{ω}-nitro-L-arginine methyl ester (L-NAME, 300 μM) in the absence (○) or in the presence (●) of (a) tetraethylammonium (10 mM), (b) tetrabutylammonium (1 mM) or (c) charybdotoxin (50 nM) in human small omental arteries. The level of pre-contraction induced by U46619 was (g) 1.30±0.2 (control), 1.31±0.18 (tetraethylammonium), 1.32±0.13 (tetrabutylammonium) and 1.29±0.22 (charybdotoxin). The points are mean±s.e.mean (vertical lines) of 5–7 experiments. **$P<0.01$ significantly different as compared to controls.

Limited information is available in the literature regarding the role of cyclo-oxygenase products and NO in endothelium-dependent vasorelaxation of human arteries. In general, the

experiments were performed in the presence of indomethacin, precluding determination of the role of PGI_2. However, the involvement of NO in endothelium-dependent relaxation has been demonstrated in human peripheral and cerebral arteries (Woolfson & Poston, 1990; Nakashima *et al*., 1993; Deng *et al*., 1995; Petersson *et al*., 1995). The above results show that, in human omental arteries, it was necessary to block both the NO pathway (by L-NAME or Hb) and the cyclo-oxygenase pathway to reduce the BK-induced relaxation. In addition, the combination of L-NAME and indomethacin produced contraction in the absence of any constrictor agent. The failure of L-NAME, Hb and indomethacin alone to produce any alteration of the effect of BK was not likely to be due to their inability to block NO production or effect, or cyclo-oxygenase activity, respectively, as revealed by the experiments in which they were added simultaneously. The identical effects of L-NAME and Hb, in the presence of indomethacin, and their lack of additivity, suggest that the NO pathway was actually blocked by both L-NAME and Hb in the experimental conditions used. At the high concentrations used in the present study, the inhibitors are able to inhibit entirely the two pathways in many vascular preparations. The results suggest that simultaneous inhibition of production of both NO and cyclo-oxygenase derived prostaglandin(s) was required to reveal their involvement. Similar results were recently found in human saphenous vein (Barker *et al*., 1996). It seems that a prostaglandin, probably PGI_2, is able to substitute for the lack of NO, and *vice versa*, in endothelium-dependent vasorelaxation of human omental small arteries. Further studies are needed to understand better the interaction between endothelial relaxant factors, but the present results suggest that an interaction similar to that seen in the human saphenous vein probably occurs in human omental resistance arteries. Furthermore, the existence of basal release of both NO and a relaxing prostaglandin in human isolated arteries has not been found previously.

The finding that the relaxing effects of A23187 and THAPS were abolished by indomethacin plus L-NAME, whereas the relaxation due to an equiactive concentration of BK was not, is also consistent with the existence of an additional mechanism in BK-induced vasorelaxation. Contrary to A23187 and THAPS, which produce an increase in $[Ca^{2+}]_i$ in endothelial cells via direct action on Ca^{2+} handling (Chen & Suzuki, 1990; Busse *et al*., 1993), BK acts via second messengers (Derian & Moskowitz, 1986; Lambert *et al*., 1986). One can therefore hypothesize that activation of second messenger(s) is required for triggering the indomethacin plus L-NAME resistant component of the response to BK in human omental arteries.

The existence of an indomethacin plus L-NAME (or Hb) resistant component of vasorelaxation indicates the involvement of NO and cyclo-oxygenase-independent mechanism(s). The following evidence supports the implication of EDHF: in the presence of indomethacin plus L-NAME, the response to BK was abolished by 40 mM KCl depolarization and it was partially inhibited by the K^+ channel blocker, TBA. Exposure of vessels to 40 mM KCl-PSS may not only inhibit the effect of EDHF on smooth muscle cells, but also decrease the release of vasoactive factors from endothelial cells. However, in the presence of indomethacin plus L-NAME, its effect could not be due to impaired release of cyclo-oxygenase derivative(s) and NO, as the two pathways were already blocked. Furthermore, the relaxation induced by the NO-donor SNAP was not significantly affected in 40 mM KCl-PSS containing L-NAME plus indomethacin. This is consistent with previous data showing that exposure to high extracellular K^+ concentration does not affect NO-mediated relaxations in a number of blood vessels (Plane *et al*., 1992; Plane & Garland, 1993) and does not impair the increase in tissue guanosine 3′:5′-cyclic monophosphate (cyclic GMP) induced by BK in pig coronary arteries (Kühberger *et al*., 1994).

Hyperpolarization and subsequent relaxation produced by EDHF involve the activation of K^+ channels in vascular smooth muscle cells (Garland *et al*., 1995). Therefore we tested several K^+ channel blockers. Among them, only TBA, an inhibitor of BK_{Ca}, partially reduced the L-NAME plus indomethacin resistant relaxation. This is in accordance with the results of previous studies conducted in a number of arteries from animals showing that part of the action of EDHF involves the activation of BK_{Ca} (Chen *et al*., 1991; Cowan *et al*., 1993; Högestätt & Zygmunt, 1996). However, the results obtained here with other K^+ channel blockers are in contrast with data indicating that EDHF can activate apamin-, glibenclamide- and 4-aminopyridine-sensitive channels, respectively SK_{Ca}, K_{ATP} and K_V (Plane & Garland, 1993; Cowan *et al*., 1993; Murphy & Brayden, 1995). Furthermore, TEA and CTX, which are BK_{Ca} blockers, failed to inhibit the response to BK. The reason for the lack of effect of TEA might be its efficacy in relation to its low selectivity for BK_{Ca} (Edwards & Weston, 1994). Also, BK_{Ca} of vascular smooth muscle cells of human omental arteries might be less sensitive to CTX. Different results found in various vascular beds may correspond to the presence of different K^+ channels or differential mechanisms of activation by EDHF. In addition, different EDHFs might be produced in different vascular beds, with differential selectivity for the various K^+ channels.

In the present study, both the PLA_2 inhibitor, quinacrine, and the cytochrome P-450 inhibitor, SKF 525a, failed to inhibit BK-induced relaxation. Another cytochrome P-450 inhibitor, clotrimazole, partially inhibited this relaxation. However, clotrimazole has been shown to inhibit different K^+ channels of arterial myocytes, such as Ca^{2+}-activated K^+ channels (Fulton *et al*., 1994) and K_V (Yuan *et al*., 1995). These data do not favour the hypothesis that metabolites formed through the cytochrome P-450 pathway are involved in the response to BK of human small omental arteries, as suggested in other vessels (Komori & Vanhoutte, 1990; Hecker *et al*., 1994; Fulton *et al*., 1995). Nevertheless, the present results are in accordance with recent studies conducted in guinea-pig carotid artery (Corriu *et al*., 1996) showing that PLA_2 inhibitors and several inhibitors of cytochrome P-450 did not inhibit the hyperpolarization produced by acetylcholine. The existence of different EDHFs might explain the discrepancies in the literature concerning their nature.

In conclusion, BK produces an endothelium-dependent relaxation of human small omental arteries through the activation of B_2 receptors. This relaxation involves NO, cyclo-oxygenase derived prostanoid(s) and another factor with properties of EDHF. In addition, NO and cyclo-oxygenase derivative(s) are able to substitute for each other both in basal and BK-induced endothelium-dependent relaxation, when one of two pathways is blocked. A TBA-sensitive mechanism, probably opening of BK_{Ca}, is implicated in the EDHF-like component of relaxation to BK. By contrast with BK, NO plus cyclo-oxygenase product(s) entirely account for endothelium-dependent relaxations elicited by agents acting on Ca^{2+} handling (A23187 for THAPS). This suggests a role for intracellular messengers other than Ca^{2+} in the generation of the EDHF-like component of BK-induced relaxation. Finally, the results do not support the hypothesis that a cytochrome P-450 product is involved in the vasorelaxation effect of BK in human small omental arteries.

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