British Journal of Pharmacology (2001) 133, 902–908

www.nature.com/bjp

Endothelium-derived hyperpolarizing factor and potassium use different mechanisms to induce relaxation of human subcutaneous resistance arteries

[1]C.-A. McIntyre, [1]C.H. Buckley, [1]G.C. Jones, [1]T.C. Sandeep, [1]R.C. Andrews, [2]A.I. Elliott, [3]G.A. Gray, [1]B.C. Williams, [2]J.A. McKnight, [1]B.R. Walker & *,[1]P.W.F. Hadoke

[1]Department of Medical Sciences, Western General Hospital, University of Edinburgh, Edinburgh; [2]Metabolic Unit, Western General Hospital, University of Edinburgh, Edinburgh and [3]Department of Biomedical Sciences, Hugh Robson Building, University of Edinburgh, Edinburgh

1 This investigation examined the hypothesis that release of K^+ accounts for EDHF activity by comparing relaxant responses produced by ACh and KCl in human subcutaneous resistance arteries.

2 Resistance arteries (internal diameter 244 ± 12 μm, $n=48$) from human subcutaneous fat biopsies were suspended in a wire myograph. Cumulative concentration-response curves were obtained for ACh ($10^{-9}-3\times10^{-5}$ M) and KCl (2.5–25 mM) following contraction with noradrenaline (NA; 0.1–3 μM).

3 ACh (E_{max} $99.07 \pm 9.61\%$; $-\text{LogIC}_{50}$ 7.03 ± 0.22; $n=9$) and KCl (E_{max} $74.14 \pm 5.61\%$; $-\text{LogIC}_{50}$ 2.12 ± 0.07; $n=10$)-induced relaxations were attenuated ($P<0.0001$) by removal of the endothelium (E_{max} $8.21 \pm 5.39\%$ and $11.56 \pm 8.49\%$, respectively; $n=6-7$).

4 Indomethacin (10 μM) did not alter ACh-induced relaxation whereas L-NOARG (100 μM) reduced this response (E_{max} $61.7 \pm 3.4\%$, $P<0.0001$; $n=6$). The combination of ChTx (50 nM) and apamin (30 nM) attenuated the L-NOARG-insensitive component of ACh-induced relaxation (E_{max}: $15.2 \pm 10.5\%$, $P<0.002$, $n=6$) although these arteries retained the ability to relax in response to 100 μM SIN-1 (E_{max} $127.6 \pm 13.0\%$, $n=3$). Exposure to $BaCl_2$ (30 μM) and Ouabain (1 mM) did not attenuate the L-NOARG resistant component of ACh-mediated relaxation (E_{max}, 76.09 ± 8.92, $P=0.16$; $n=5$).

5 KCl-mediated relaxation was unaffected by L-NOARG + indomethacin (E_{max}; $68.1 \pm 5.6\%$, $P=0.33$; $n=5$) or the combination of L-NOARG/indomethacin/ChTx/apamin (E_{max}; $86.61 \pm 14.02\%$, $P=0.35$; $n=6$). In contrast, the combination of L-NOARG, indomethacin, ouabain and $BaCl_2$ abolished this response (E_{max}, $5.67 \pm 2.59\%$, $P<0.0001$, $n=6$).

6 The characteristics of KCl-mediated relaxation differed from those of the nitric oxide/prostaglandin-independent component of the response to ACh, and were endothelium-dependent, indicating that K^+ does not act as an EDHF in human subcutaneous resistance arteries.

British Journal of Pharmacology (2001) **133,** 902–908

Keywords: Endothelium-dependent relaxation; endothelium-derived hyperpolarizing factor; nitric oxide; potassium channels; human resistance arteries

Abbreviations: ACh, acetylcholine; BSA, bovine serum albumin; ChTx, charybdotoxin; EDHF, endothelium-derived hyperpolarizing factor; EDTA, ethylene diamine tetraacetic acid; F, Female; KPSS, high potassium physiological salt solution; L-NOARG, N^G-nitro-L-arginine; M, Male; NA, noradrenaline; NO, nitric oxide; PG, prostaglandin; PSS, physiological salt solution; SIN-1, 3′-morpholinosydnonimine

Introduction

The vascular endothelium modulates agonist-dependent relaxation by releasing substances such as nitric oxide (NO) and prostaglandins (PGs) (Furchgott & Vanhoutte, 1989). In some vessels, particularly those with a small diameter (Shimokawa *et al*., 1996), a component of the endothelium-dependent relaxation is insensitive to nitric oxide synthase and cyclooxygenase inhibition (Nagao *et al*., 1992; Brandes *et al*., 1997). This component appears to be mediated by hyperpolarization of the vascular smooth muscle cells (Brayden, 1990), suggesting the existence of a distinct endothelium-derived hyperpolarizing factor (EDHF) (Taylor & Weston, 1988; Feletou & Vanhoutte, 1997).

The identity of EDHF has yet to be confirmed, although activity of this factor has been attributed to epoxyeicosatrienoic acids (Hecker *et al*., 1994), endocannabinoids (Randall *et al*., 1996), hydrogen peroxide (Matoba *et al*., 2000) and the presence of myoendothelial gap junctions (Chaytor *et al*., 1998). A recent study suggested that release of K^+ into the myoendothelial space accounted for EDHF activity in rat hepatic and mesenteric arteries (Edwards *et al*., 1998). In this study, EDHF-mediated responses (but not those to exogenous K^+) were inhibited by using charybdotoxin (ChTx) and apamin to block large (BK_{Ca}) and small (SK_{Ca}) conductance calcium-activated potassium channels on

*Author for correspondence at: Department of Medical Sciences, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU; E-mail: phadoke@srv0.med.ed.ac.uk

the endothelium. In contrast, the combination of barium and ouabain inhibited responses to K^+ as well as to EDHF, suggesting that both EDHF and K^+ cause smooth muscle cell hyperpolarization through activation of inward rectifier potassium channels (K_{IR}) and Na^+/K^+-ATPases. Subsequent studies have, however, challenged the identification of K^+ as EDHF by demonstrating differences in the characteristics of EDHF and K^+-induced relaxation in rat mesenteric (Doughty *et al*., 2000; Lacy *et al*., 2000), porcine coronary and guinea-pig carotid arteries (Quignard *et al*., 1999).

Human resistance arteries have been used extensively to examine the cardiovascular defects associated with the development of a variety of different disease processes. A large component of endothelium-dependent relaxation in these arteries is mediated by EDHF (Nakashima *et al*., 1993; Urakami-Harasawa *et al*., 1997; Wallerstedt & Bodelsson, 1997) but the mechanism of this response has not been elucidated. This investigation aimed to determine whether K^+ accounted for EDHF activity in human subcutaneous resistance arteries by comparing the NO-independent, PG-independent component of ACh-induced relaxation with relaxant responses produced by exogenous potassium.

Methods

Vessel preparation

Biopsies of gluteal skin and subcutaneous fat (2 cm × 1 cm × 1 cm) were obtained under local anaesthesia (2% lignocaine hydrochloride; Astra, Herts, U.K.) from 26 healthy volunteers (20 Male, six Female; age 46 ± 3 years). Written informed consent and approval from the Lothian Research Ethics Committee were obtained. Each biopsy was immersed immediately in cold (4°C) physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 24, KH_2PO_4 1.18, K_2EDTA 0.026 and D-glucose, 5.5. Dissection of these biopsies provided 48 resistance artery sections (mean internal diameter 244 ± 12 μm) for pharmacological analysis. Ring segments of these arteries, 2 mm in length, were suspended on two 40 μm stainless steel wires in a small vessel myograph for measurement of isometric force. The myograph bath contained PSS maintained at 37°C and perfused with 95% O_2/5% CO_2. Following an equilibration period of 30 min, the resting tension-internal circumference relationship was determined by stepwise radial stretching and the vessels were set to their optimum resting level (0.9 L_{100}, where L_{100} is the internal circumference the vessels would have when relaxed and subjected to a pressure of 100 mmHg; Mulvany & Halpern, 1977). After equilibration for a further 30 min, vessel viability was assessed using a standard start procedure (Aalkjaer *et al*., 1987). This consisted of five consecutive stimulations lasting 3 min, each followed by a 5 min washout period. The first, second and fifth contractions were produced using a high (125 mM) potassium solution (KPSS; made by equimolar substitution of KCl for NaCl in PSS) containing 10 μM noradrenaline (NA). The third was obtained with NA (10 μM) alone and the fourth with KPSS alone. The functional integrity of the endothelium was assessed by adding ACh (0.1 – 10 μM) to vessels contracted with sufficient NA (0.1 – 3 μM) to produce 60 – 80% of the response KPSS.

The contribution of EDHF to ACh-mediated relaxation

Sixteen resistance arteries (internal diameter 183 ± 15 μm) from 14 male subjects (age 57 ± 12 years) were used for this part of the investigation. After the standard start procedure, a cumulative concentration-response curve to ACh (0.001 – 300 μM) was obtained following precontraction with a sub-maximal concentration (0.1 – 3 μM) of NA (to produce a contraction of ~60 – 80% the maximum response to KPSS). The artery was washed with PSS (37°C) and the procedure repeated following incubation with either; (a) indomethacin (10 μM for 45 min, $n = 6$), (b) N^G-nitro-L-Arginine (L-NOARG; 100 μM for 45 min, $n = 6$), or (c) L-NOARG (100 μM for 45 min), plus charybdotoxin (ChTx; 50 nM for 10 min) and apamin (30 nM for 10 min, $n = 6$). Arteries were exposed to only one antagonist except for two of those initially incubated with indomethacin which were subsequently exposed to the combination L-NOARG + ChTx + apamin. Three of the arteries incubated with L-NOARG + ChTx + apamin, were also exposed to a single concentration (100 μM) of the exogenous NO donor, 3′-morpholinosydnonimine (SIN-1) once the concentration-response curve to ACh had been completed.

Comparison of K^+-induced relaxation with the EDHF-mediated component of ACh-evoked relaxation

Thirty-two resistance arteries (internal diameter 273 ± 14 μm) obtained from 12 subjects (six male, six female; age 32 ± 4 years) were used for this part of the investigation. The endothelium was removed from some arteries by rubbing the luminal surface with a single hair. Cumulative concentration-response curves were obtained using ACh (0.001 – 300 μM) and KCl (2.5 – 25 mM), in intact ($n = 9 – 10$) and denuded ($n = 6 – 7$) arteries, after pre-contraction (to produce a contraction of ~60 – 80% the maximum response to KPSS) with a sub-maximal concentration of NA (0.1 – 3 μM). Responses to KCl were repeated following incubation with a combination of either (a) L-NOARG (100 μM) + indomethacin (10 μM; 45 min, $n = 5$); (b) L-NOARG (100 μM) + indomethacin (10 μM for 45 min) plus charybdotoxin (ChTx; 50 nM for 10 min) and apamin (30 nM for 10 min, $n = 6$) or (c) L-NOARG (100 μM) + indomethacin (10 μM for 45 min) plus $BaCl_2$ (30 μM for 10 min) and ouabain (1 mM for 10 min, $n = 6$). Concentration-response curves to ACh were also produced in the arteries exposed to the combinations described for groups (b) and (c).

Drugs

All salts were obtained from BDH Laboratory supplies, (Poole, Dorset, U.K). All drugs were purchased from Sigma, (Poole, Dorset, U.K.), except for 3′ morpholinosydnonimine, charybdotoxin and apamin which were obtained from Alexis Corporation Ltd (Nottingham, U.K.). Acetylcholine chloride, ouabain, barium chloride and noradrenaline bitartrate were dissolved in distilled water; indomethacin in 1.5×10^{-3} M Na_2CO_3 (final bath concentration of Na_2CO_3 did not exceed 0.015 mM) and apamin in 0.05 M acetic acid (final bath

concentration of acetic acid did not exceed 0.15 mM). Charybdotoxin was dissolved in a Tris buffer (10 mM, pH 7.5) containing 0.1% BSA, 100 mM NaCl and 1 mM EDTA (final bath concentrations of NaCl and EDTA did not exceed 5 and 0.05 mM, respectively). 0.01% BSA was added to the myograph chamber before applying the toxins. Stock solutions were stored at −20°C, thawed as required and subsequent dilutions made in distilled water. The concentrations quoted are final molar concentrations in the organ bath.

Statistics

All values are presented as mean ± standard error mean (s.e.mean) from n experiments (where *n* represents the number of subjects). Relaxation responses to ACh and KCl are expressed as a percentage of the initial NA-induced precontraction. The concentration of agonist required to produce 50% of the maximum response (IC_{50}) was obtained by fitting the Hill equation to the data using curve fitting software (Fig. P, Biosoft, Cambridge, U.K.) and is expressed as the negative logarithm of the IC_{50} ($-\log IC_{50}$). Comparisons of maximum relaxation and $-\log IC_{50}$ values were made using Student's paired or unpaired *t*-test, as appropriate, and significance was assumed when $P<0.05$.

Results

The contribution of EDHF to ACh-induced relaxation

ACh caused approximately 80–100% relaxation in intact human subcutaneous resistance arteries following pre-contraction with a sub-maximal concentration of NA (0.1–3 μM; Figure 1). None of the inhibitors caused an increase in either the resting tone of the arteries or the response to the pre-contracting concentration of NA.

Incubation with indomethacin (Figure 1a) did not alter the magnitude (E_{max}, 97.56 ± 1.83%, $n=6$) or sensitivity ($-\log IC_{50}$, 7.24 ± 0.20, $n=6$) of ACh-evoked relaxation when compared with controls (90.80 ± 4.69%, $P=0.18$ and 7.23 ± 0.25 $P=0.96$, respectively; $n=6$). In contrast, exposure to L-NOARG (Figure 1b) resulted in a significant ($P<0.0001$), although not total, reduction in maximum relaxation (61.68 ± 3.38%, $n=6$) compared with controls (91.55 ± 3.95%, $n=6$) with a corresponding reduction in sensitivity ($-\log IC_{50}$, 6.41 ± 0.10 vs 7.19 ± 0.13, respectively, $P<0.005$; $n=6$). Arteries exposed to the combination of L-NOARG plus ChTx and apamin demonstrated almost total attenuation of ACh-mediated relaxation (E_{max}, 15.2 ± 10.5%, $n=6$) despite producing a full concentration-response curve before exposure to these inhibitors (E_{max}, 92.59 ± 3.65%, $P<0.002$; $-\log IC_{50}$, 7.70 ± 0.30, $n=6$). These arteries maintained their ability to relax in response to exogenous NO, as SIN-1 (100 μM) caused complete relaxation in the presence of L-NOARG, ChTx and apamin (127.6 ± 13.0%; $n=3$).

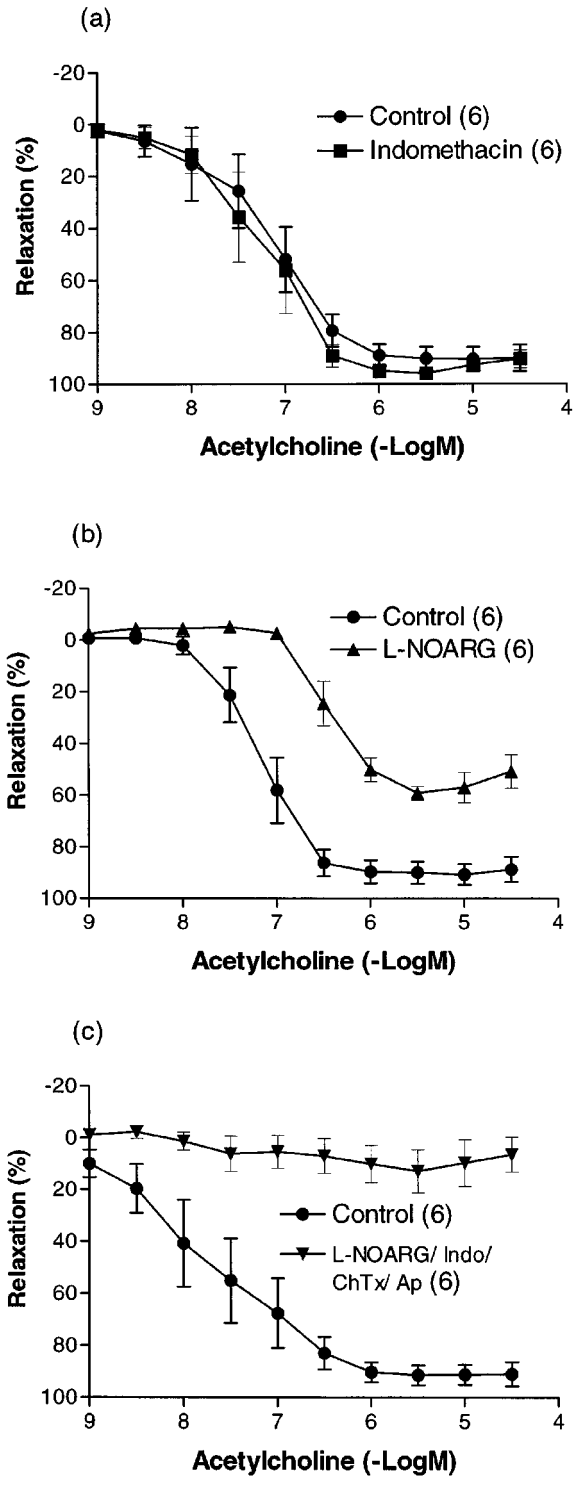

Figure 1 Cumulative concentration-response curves to ACh (10^{-9}–3×10^{-5} M) before and after incubation with the following combination of inhbitors (a) the cyclooxygenase inhibitor indomethacin (10 μM for 45 min), (b) the NO synthase inhibitor L-NOARG (100 μM for 45 min) or (c) L-NOARG (100 μM for 45 min) plus the K^+ channel blockers ChTx (50 nM for 10 min) and apamin (30 nM for 10 min). Results are shown as mean ± s.e.mean, for (*n*) arteries.

Comparison of K^+-induced relaxation with the EDHF-mediated component of ACh-evoked relaxation

Relaxation responses were obtained using potassium in 10 arteries with an intact endothelium and responses to ACh were also tested in nine of these. Typical relaxation responses were obtained with ACh (E_{max}, 99.07 ± 9.61%; $-\log IC_{50}$, 7.03 ± 0.224; $n=9$), which produced a sustained concentration-dependent relaxation (Figure 2). In contrast, although

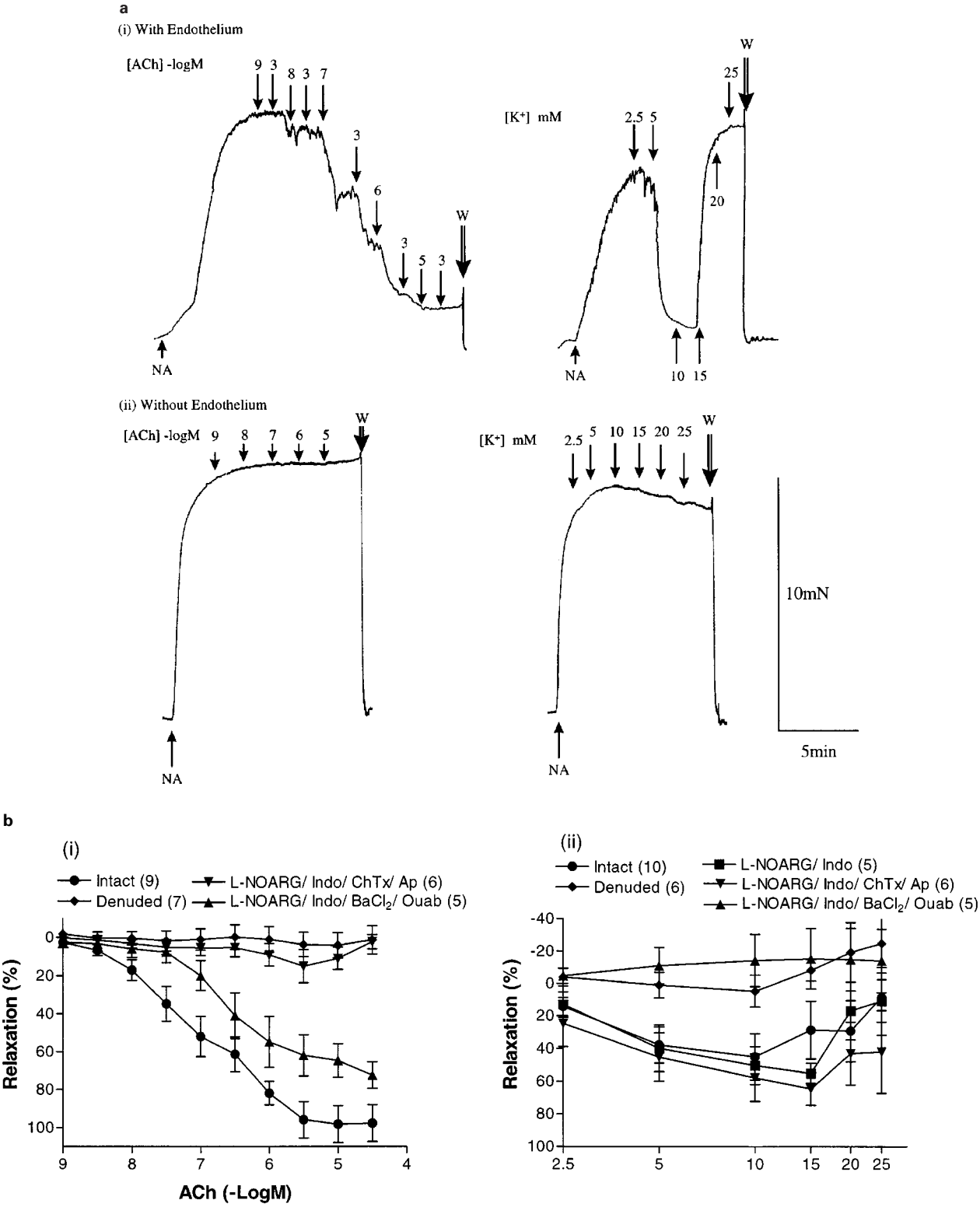

Figure 2 Comparison of ACh- and KCl-mediated relaxation. (a) Representative traces showing (i) relaxation responses of an intact artery to acetylcholine and KCl and (ii) the effect of removal of the endothelium on these responses. (b) Cumulative concentration-response curves for (i) acetylcholine and (ii) KCl obtained in arteries with and without an intact endothelium or in the presence of L-NOARG (100 μM) and indomethacin (10 μM) alone or combined with either ChTx (50 nM) and apamin (30 nM) or $BaCl_2$ (30 μM) plus ouabain (1 mM). Results are shown as mean$\pm$s.e.mean, for (n) arteries.

potassium also relaxed these resistance arteries (E_{max}, 74.14±5.61%, IC_{50}, 6.09±1.17 mM; $-\log IC_{50}$, 2.12±0.07, $n=10$), the response to this compound (Figure 2) was inconsistent and was superseded by a reversal of the initial relaxation response as the concentration of KCl rose (>15–25 mM). As expected, removal of the endothelium virtually abolished responses to ACh (13.34±6.16%, $n=7$, $P<0.0001$) but also abolished potassium-mediated relaxation (E_{max}, 15.53±9.18%, $n=6$, $P<0.001$) (Figure 2).

The potassium-induced relaxation was not affected by incubation with L-NOARG and indomethacin (E_{max}, 68.1±5.6%, $P=0.51$; IC_{50}, 5.74±1.86 mM; $-\log IC_{50}$, 2.34±0.15, $P=0.33$, $n=5$) or with the combination of L-NOARG with indomethacin, ChTx and apamin (E_{max}, 86.61±14.02%; $P=0.35$; IC_{50}, 6.78±2.90 mM; $-\log IC_{50}$, 2.68±0.52, $P=0.23$, $n=6$). Indeed the maximum relaxation evoked by potassium tended to be larger in the latter group. Exposure of vessels to the combination of $BaCl_2$ and ouabain resulted in an increase in basal tone of 0.40±0.17 mN (equivalent to 16.6±7.4% of the maximum response to KPSS; $n=11$). This tended to be larger in arteries used for producing responses to ACh (22.4±17.6% KPSS; $n=5$) than in those subsequently exposed to KCl (12.0±5.6% KPSS; $n=6$). Once this contraction had stabilized, vessels were contracted with sufficient NA (0.1–3 μM) to produce a contraction 60–80% the size of the maximum response to KPSS (responses to ACh obtained in one artery were discarded as the combination of $BaCl_2$ plus ouabain produced a contraction equivalent to 80% of the response to KPSS). Potassium-induced relaxation was totally abolished by incubation with the combination of L-NOARG with indomethacin, $BaCl_2$ and ouabain (5.7±2.6%; $n=6$; $P<0.0001$). In contrast, a considerable ACh-induced relaxation remained evident following exposure to this combination of inhibitors although there was a trend towards reduced relaxation that did not achieve significance (E_{max}, 76.09±8.92%; $P=0.16$; $-\log IC_{50}$, 6.47±0.23; $P=0.11$, $n=5$).

Discussion

Previous investigations have demonstrated that an NO/PG-independent component of ACh-evoked relaxation is mediated by EDHF (Nakashima *et al*., 1993; Urakami-Harasawa *et al*., 1997; Wallerstedt & Bodelsson, 1997). Studies in arteries from experimental animals have suggested that K^+ accounts for EDHF activity (Edwards *et al*., 1998). In order to clarify whether K^+ acts as an EDHF in human arteries, this investigation compared potassium-induced and EDHF-induced relaxation responses in subcutaneous resistance arteries isolated from biopsies of gluteal fat. The characteristics of potassium-induced relaxation were different from the EDHF-mediated response and, of significance, were abolished by removal of the endothelium. Taken together, this suggests that release of endothelium-derived K^+ into the myoendothelial space does not account for EDHF activity in human subcutaneous resistance arteries.

Comparison with previous investigations indicates that the ChTx/ apamin-sensitive, NO-independent component of ACh-evoked relaxation is mediated by EDHF. In rat mesenteric arteries contracted with an α-adrenoceptor agonist, the NO-independent component of ACh-mediated relaxation was caused by smooth muscle cell hyperpolarisation (Plane & Garland, 1996). This response is abolished by the combination of ChTx and apamin (Zygmunt & Högestätt, 1996), probably by inhibition of BK_{Ca} and SK_{Ca} on the endothelium (Doughty *et al*., 1999). The persistence of a significant NO-independent (EDHF-mediated) relaxation in response to ACh is consistent with previous studies of human subcutaneous (Woolfson & Poston, 1990; Deng *et al*., 1995; Hillier *et al*., 1998), omental (Ohlmann *et al*., 1997), gastroepiploic (Urakami-Harasawa *et al*., 1997), coronary (Nakashima *et al*., 1993) and pial (Petersson *et al*., 1995) arteries. Incomplete inhibition is unlikely to account for residual relaxation as a lower concentration of L-NOARG (3×10^{-5} M) abolished ACh-induced, endothelium-dependent relaxation in the rat aorta, pulmonary and iliac arteries (Nagao *et al*., 1992). Furthermore, incomplete inhibition of ACh-mediated relaxation was not overcome by increasing the concentration of L-NOARG (100–300 μM; Brandes *et al*., 1997) or by the combined application of two different L-arginine analogues (Plane & Garland, 1996; Plane *et al*., 1997). The failure of indomethacin to attenuate ACh-mediated relaxation in the present study confirms that prostanoids do not contribute to this response in the human gluteal, subcutaneous resistance artery. This is also consistent with previous studies, in our own and other laboratories, in which indomethacin was shown to have no effect on ACh-or bradykinin-mediated relaxation of human gluteal resistance arteries when applied alone or in combination with NO synthase inhibitors (Hillier *et al*., 1998; Buckley *et al*., 1999). The mechanism of endothelium-dependent relaxation of human resistance arteries may depend upon the origin of a particular vessel, however, as bradykinin-mediated relaxation of human omental arteries has an indomethacin-sensitive component which becomes evident in the presence of an NO inhibitor (Ohlmann *et al*., 1997).

The ability of exogenous potassium to relax human gluteal resistance arteries compares with results obtained in resistance arteries from experimental animals (Edwards *et al*., 1998); Quignard *et al*., 1999; Doughty *et al*., 2000; Lacy *et al*., 2000). The identification of K^+ as an EDHF in the earlier study was based on a comparison with the NO/PG-independent component of the response to ACh (Edwards *et al*., 1998); responses to both ACh and exogenous K^+ were abolished by inhibition of K_{IR} and Na^+/K^+ ATPase, indicating a common mechanism. Exogenous K^+, however, produced an endothelium-independent hyperpolarization of smooth muscle cells that was unaffected by the combination of ChTx and apamin. This is consistent with ACh stimulating release of K^+ from endothelial cells *via* ChTx/ apamin-sensitive channels. In the present study, however, the characteristics of potassium-induced and EDHF-mediated relaxation were different: whereas the ACh-induced relaxation was highly reproducible and sustained, relaxation responses to potassium were more variable and reversed readily at higher K^+ concentrations. This is consistent with a recent study showing that exogenous K^+ will only produce a reproducible, sustained relaxation of rat resistance arteries if they are bathed in a Kreb's solution lacking K^+ ions (Lacy *et al*., 2000). More striking, however, was the demonstration that, as in the rat mesenteric (Lacy *et al*., 2000) and renal (Jiang & Dusting, 2001) arteries, potassium-mediated relaxa-

tion of human subcutaneous arteries was abolished by removal of the endothelium. This indicates an obligatory role for the endothelium in K^+-mediated relaxation, suggesting that it may be mediated by a further endothelium-derived factor or is dependent upon myoendothelial gap junctions (Doughty *et al*., 2000). Finally, the inability of barium and ouabain to inhibit ACh-mediated relaxation, whilst abolishing responses to potassium, indicated that these compounds caused relaxation *via* different mechanisms. This observation contrasts with the study by Edwards *et al*. (1998) but is consistent with data obtained in subsequent investigations (Quignard *et al*., 1999; Lacy *et al*., 2000). The ability of K^+ to relax only a proportion ($\sim$30%) of rat mesenteric resistance arteries in one study (Doughty *et al*., 2000) suggests that this response may even vary in different regions of the same artery, possibly reflecting variations in K_{IR} and Na^+/K^+-ATPase activity (Albarwani *et al*., 1995).

In conclusion, this investigation demonstrated an NO/PG-independent response to ACh in human subcutaeous arteries which had characteristics consistent with EDHF-mediated relaxation. The failure of exogenous potassium to produce an endothelium-independent relaxation which mimicked this response indicates that potassium is not an EDHF in these vessels.

This work was supported by a project grant from The British Diabetic Association (Diabetes, U.K.). P. Hadoke is a BHF-funded Resarch Fellow and B.R. Walker is a British Heart Foundation Senior Research Fellow.

References

AALKJAER, C., HEAGERTY, A.M., PETERSEN, K.K., SWALES, J.D. & MULVANY, M.J. (1987). Evidence for increased media thickness, increased neuronal amine uptake and depressed excitation-contraction coupling in isolated resistance vessels from essential hypertensives. *Circ. Res.,* **61,** 181 – 186.

ALBARWANI, S., HEINERT, G., TURNER, J.L. & KOZLOWSKI, R.Z. (1995). Differential K^+ channel distribution in smooth muscle cells isolated from the pulmonary artery tree of the rat. *Biochem. Biophys. Res. Commun.,* **208,** 183 – 189.

BRANDES, R.P., BEHRA, A., LEBHERZ, C., BÖGER, R.H., BODE-BÖGER, S.M., PHIVTHONG-HGAM, L. & MÜGGE, A. (1997). NG-nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation in the rabbit renal artery: effect of hypercholesterolemia. *Atherosclerosis,* **135,** 49 – 55.

BRAYDEN, J.E. (1990). Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilation. *Am. J. Physiol.,* **259,** H668 – H673.

BUCKLEY, C., HADOKE, P.W.F., HENRY, E. & O'BRIEN, C.J. (1999). Altered balance of endothelium-derived relaxing factors in the acetylcholine-mediated relaxation of subcutaneous arteries isolated from patients with normal pressure glaucoma. *Br. J. Pharmacol.,* **128,** (Proc Suppl), P264.

CHAYTOR, A.T., EVANS, W.H. & GRIFFITH, T.M. (1998). Central role of heterocellular gap junctional communication in endothelium-dependent relaxations of rabbit arteries. *J. Physiol.,* **508,** 561 – 573.

DENG, L.-Y., LI, J.-S. & SCHIFFRIN, L. (1995). Endothelium-dependent relaxation of small arteries from essential hypertensive patients: mechanisms and comparison with normotensive subjects and with responses of vessels from spontaneously hypertensive rats. *Clin. Sci.,* **88,** 611 – 622.

DOUGHTY, J.M., BOYLE, J.P. & LANGTON, P. (2000). Potassium does not mimic EDHF in rat mesenteric arteries. *Br. J. Pharmacol.,* **130,** 1174 – 1182.

DOUGHTY, J.M., PLANE, F. & LANGTON, P.D. (1999). Charybdotoxin and apamin block EDHF in rat mesenteric artery if selectively applied to the endothelium. *Am. J. Physiol.,* **276,** H1107 – H1112.

EDWARDS, G., DORA, K.A., GARDENER, M.J., GARLAND, C.J. & WESTON, A.H. (1998). K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature,* **396,** 269 – 272.

FELETOU, M. & VANHOUTTE, P.M. (1997). EDHF exits. *B.J. Pharmacol.,* **122,** (Suppl.), U142.

FURCHGOTT, R.F. & VANHOUTTE, P.M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB J.,* **3,** 2007 – 2018.

HECKER, M., BARA, A.T., BAUERSACHS, J. & BUSSE, R. (1994). Characterisation of endothelium-derived hyperpolarizng factor as a cytochrome P450-derived arachidonic acid metabolite in mammals. *J. Physiol.,* **481,** 407 – 414.

HILLIER, C., McGEE, A. & McGRATH, J.C. (1998). Bradykinin-induced vasorelaxation in both diabetic and non-diabetic human resistance arteries does not involve prostacyclin. *J. Vasc. Res.,* **35,** 6-6 (Suppl.1), [Abstract].

JIANG, F. & DUSTING, G.J. (2001). Endothelium-dependent vasorelaxation independent of nitric oxide and K^+ release in isolated renal arteries of rats. *Br. J. Pharmacol.,* **132,** 1558 – 1564.

LACY, P.S., PILKINGTON, G., HANVESAKUL, R., FISH, H.J., BOYLE, J.P. & THURSTON, H. (2000). Evidence against potassium as an endothelium-derived hyperpolarizing factor in rat mesenteric small arteries. *Br. J. Pharmacol.,* **129,** 605 – 611.

MATOBA, T., SHIMOKAWA, H., NAKASHIMA, M., HIIRAKAWA, Y., MUKAI, Y., HIRANO, K., KANAIDE, H. & TAKESHITA, A. (2000). Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J. Clin. Invest.,* **106,** 1521 – 1530.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.,* **41,** 19 – 26.

NAGAO, T., ILLIANO, S. & VANHOUTTE, P.M. (1992). Heterogeneous distribution of endothelium-dependent relaxations resistant to N^G-nitro-L-arginine in rats. *Am. J. Physiol.,* **263,** H1090 – H1094.

NAKASHIMA, M., MOMBOULI, J.-V., TAYLOR, A.A. & VANHOUTTE, P.M. (1993). Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. *J. Clin. Invest.,* **92,** 2867 – 2871.

OHLMANN, P., MARTINEZ, 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.

PETERSSON, J., ZYGMUNT, P.M., BRANDT, L. & HOGESTATT, E.D. (1995). Substance P-induced relaxation and hyperpolarization in human cerebral arteries. *Br. J. Pharmacol.,* **115,** 889 – 894.

PLANE, F. & GARLAND, C.J. (1996). Influence of contractile agonists on the mechanism of endothelium-dependent relaxation in rat isolated mesenteric artery. *Br. J. Pharmacol.,* **119,** 191 – 193.

PLANE, F., HOLLAND, M., WALDRON, C.J., GARLAND, C.J. & BOYLE, J.P. (1997). Evidence that anandamide and EDHF act via different mechanisms in rat isolated mesenteric arteries. *Br. J. Pharmacol.,* **121,** 1509 – 1511.

QUIGNARD, J.-F., FELETOU, M., THOLON, C., VILANE, J.-P., DUHAULT, J. & VANHOUTTE, P.M. (1999). Potassium ions and endothelium-derived hyperpolarizing factor in guinea-pig carotid and porcine coronary arteries. *Br. J. Pharmacol.,* **127,** 27 – 34.

RANDALL, M.D., ALEXANDER, S.P.H., BENNET, T., BOYD, E.A., FRY, J.R., GARDINER, S.M., KEMP, P.A., McCULLOCH, A. & KENDAL, D.A. (1996). An exogenous cannabinoid as an endothelium-derived vasorelaxant. *Biochem. Biophys. Res. Commun.,* **229,** 114 – 120.

SHIMOKAWA, H., YASUTAKE, H., FUJII, K., OWADA, M.K., NAKAIKE, R., FUKUMOTO, Y., TAKAYANAGI, T., NAGAO, T., EGASHIRA, K., FUJISHIMA, M. & TAKESHITA, A. (1996). The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. *J. Cardiovasc. Pharmacol.,* **28,** 703 – 711.

TAYLOR, S.G. & WESTON, A.H. (1988). Endothelium-derived hyperpolarizing factor : a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol. Sci.,* **9,** 272 – 274.

URAKAMI-HARASAWA, L., SHIMOKAWA, H., NAKASHIMA, M., EGASHIRA, K. & TAKESHITA, A. (1997). Importance of endothelium-derived hyperpolarizing factor in human arteries. *J. Clin. Invest.,* **100,** 2793 – 2799.

WALLERSTEDT, S.M. & BODELSSON, M. (1997). Endothelium-dependent relaxation by substance P in human isolated omental arteries and veins: relative contribution of prostanoids, nitric oxide and hyperpolarization. *Br. J. Pharmacol.,* **120,** 25 – 30.

WOOLFSON, R.G. & POSTON, L. (1990). Effect of N^G-monomethyl-L-arginine on endothelium-dependent relaxation of human subcutaneous resistance arteries. *Clin. Sci.,* **79,** 273 – 278.

ZYGMUNT, P.M. & HÖGESTÄTT, E.D. (1996). Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. *Br. J. Pharmacol.,* **117,** 1600 – 1606.

(Received October 12, 2000
Revised April 24, 2001
Accepted April 30, 2001)