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Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery

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1 In the presence of indomethacin (IM, 10μM) and N$^{\omega}$-nitro-L-arginine (L-NOARG, 0.3 mM), acetylcholine (ACh) induces an endothelium-dependent smooth muscle hyperpolarization and relaxation in the rat isolated hepatic artery. The potassium (K) channel inhibitors, tetrabutylammonium (TBA, 1 mM) and to a lesser extent 4-aminopyridine (4-AP, 1 mM) inhibited the L-NOARG/IM-resistant relaxation induced by ACh, whereas apamin (0.1-0.3 μM), charybdotoxin (0.1-0.3 μM), iberiotoxin (0.1 μM) and dendrotoxin (0.1 μM) each had no effect. TBA also inhibited the relaxation induced by the receptor-independent endothelial cell activator, A23187.

2 When combined, apamin (0.1 μM) + charybdotoxin (0.1 μM), but not apamin (0.1 μM) + iberiotoxin (0.1 μM) or a triple combination of 4-AP (1 mM) + apamin (0.1 μM) + iberiotoxin (0.1 μM), inhibited the L-NOARG/IM-resistant relaxation induced by ACh. At a concentration of 0.3 μM, apamin + charybdotoxin completely inhibited the relaxation. This toxin combination also abolished the L-NOARG/IM-resistant relaxation induced by A23187.

3 In the absence of L-NOARG, TBA (1 mM) inhibited the ACh-induced relaxation, whereas charybdotoxin (0.3 μM) + apamin (0.3 μM) had no effect, indicating that the toxin combination did not interfere with the L-arginine/NO pathway.

4 The gap junction inhibitors halothane (2 mM) and 1-heptanol (2 mM), or replacement of NaCl with sodium propionate did not affect the L-NOARG/IM-resistant relaxation induced by ACh.

5 Inhibition of Na^+/K^+-ATPase by ouabain (1 mM) had no effect on the L-NOARG/IM-resistant relaxation induced by ACh. Exposure to a K^+-free Krebs solution, however, reduced the maximal relaxation by 13% without affecting the sensitivity to ACh.

6 The results suggest that the L-NOARG/IM-resistant relaxation induced by ACh in the rat hepatic artery is mediated by activation of K-channels sensitive to TBA and a combination of apamin + charybdotoxin. Chloride channels, Na^+/K^+-ATPase and gap junctions are probably not involved in the response. It is proposed that endothelial cell activation induces secretion of an endothelium-derived hyperpolarizing factor(s) (EDHF), distinct from NO and cyclo-oxygenase products, which activates more than one type of K-channel on the smooth muscle cells. Alternatively, a single type of K-channel, to which both apamin and charybdotoxin must bind for inhibition to occur, may be the target for EDHF.

Keywords: Acetylcholine; arteries; vascular endothelium; hyperpolarization; membrane potential; nitric oxide; potassium channels

Introduction

It has been suggested that endothelium-dependent hyperpolarization underlies the NO/prostanoid-independent relaxation induced by endothelial cell activation in several blood vessels (Garland & McPherson, 1992; Nagao & Vanhoutte, 1992; Zygmunt *et al.*, 1994b). However, the cellular mechanisms responsible for endothelium-dependent hyperpolarization have not been resolved. The NO/prostanoid-independent relaxation induced by ACh or bradykinin has been shown to be inhibited by charybdotoxin (inhibitor of large conductance calcium-activated K-channels) in the rabbit abdominal aorta and carotid artery, and rat isolated perfused heart (Cowan *et al.*, 1993; Fulton *et al.*, 1994), iberiotoxin (inhibitor of large conductance calcium-activated K-channels) in rat isolated small mesenteric arteries (Olesen & Hansen, 1995) and apamin (inhibitor of small conductance calcium-activated K-channels) in the rat perfused mesenteric vascular bed (Adeagbo & Triggle, 1993). It was suggested that activation of ATP-sensitive K-channels mediated the ACh-induced hyperpolarization in the rabbit middle cerebral artery and the NO/prostanoid-independent relaxation in the rabbit abdominal aorta, since these responses were inhibited by the ATP-sensitive K-channel blocker, glibenclamide (Brayden, 1990; Cowan *et al.*, 1993). However, several other studies have been unable to show an effect of glibenclamide on endothelium-dependent hyperpolarization and/or relaxation/vasodilatation (Chen *et al.*, 1991; McPherson & Angus, 1991; Eckman *et al.*, 1992; Illiano *et al.*, 1992; Nakashima *et al.*, 1993; Fulton *et al.*, 1994; Kitagawa *et al.*, 1994; Zygmunt *et al.*, 1994b; Olesen & Hansen, 1995). These apparently conflicting results may indicate that several types of K-channel are involved in endothelium-dependent hyperpolarization and relaxation.

Stimulation of the electrogenic Na^+/K^+-pump has also been suggested to mediate endothelium-dependent hyperpolarization. This is based on the observtion that ouabain and/or potassium substitution inhibited the endothelium-dependent hyperpolarization and/or relaxation induced by ACh in canine coronary, feline cerebral and rabbit renal arteries (Feletou & Vanhoutte, 1988; Brayden & Wellman, 1989; Kitagawa *et al.*, 1994). However, the endothelium-dependent hyperpolarization induced by ACh was unaffected by ouabain in the rabbit ear, and canine and guinea-pig coronary arteries (Suzuki, 1988; Chen *et al.*, 1989; 1991).

Several agonists eliciting endothelium-dependent smooth muscle hyperpolarization also hyperpolarize the endothelial cell, presumably by opening of calcium-activated K-channels (see, Bény & von der Weid, 1991; Chen & Cheung, 1992;

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Groschner *et al.*, 1992; Busse *et al.*, 1993; Sharma & Davis, 1994). Together with ultrastructural evidence for myoendothelial couplings (Spagnoli *et al.*, 1982; Taugner *et al.*, 1984; Kristek & Gerová, 1992), these observations may support the hypothesis that the hyperpolarization is initiated in the endothelium and conducted to the underlying smooth muscle via low resistance electrical couplings or gap junctions (see, Bény & von der Weid, 1991). In support of an electrotonic conduction, the gap junction uncoupler, 1-heptanol, attenuated the N^{ω}-nitro-L-arginine (L-NOARG)/indomethacin (IM)-resistant relaxation induced by bradykinin in porcine coronary arteries (Kühberger *et al.*, 1994). However, several other studies have indicated that a paracrine endothelium-derived hyperpolarizing factor (EDHF), not requiring myoendothelial gap junctions, is acting on the vascular smooth muscle cells (Feletou & Vanhoutte, 1988, Kauser *et al.*, 1989; Chen *et al.*, 1991; Bény & Pacicca, 1994; Plane & Garland, 1994).

In the rat hepatic artery, ACh induces an endothelium-dependent relaxation, which is mediated by a NO/prostanoid-independent smooth muscle hyperpolarization as well as by NO (Zygmunt *et al.*, 1994a, b). The aim of the present study was to investigate the role of potassium and chloride channels, the Na^+/K^+-pump and gap junctions in the L-NOARG/IM-resistant relaxation in this artery.

Methods

Experimental procedure

The hepatic artery was removed from female Sprague-Dawley rats (250–300 g). The artery was cut into ring segments, 1–2 mm in length, which were suspended between two metal prongs in organ baths (2.5 ml), containing Krebs solution of the following composition (mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.5 and glucose 11. The solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at a temperature of 37°C, resulting in a pH of 7.4. During an equilibration period of about 1 h, the vessels were repeatedly stretched until a stable resting tension of approximately 2 mN mm^{-1} vessel length was obtained. 'Isometric' tension was measured by a Grass Instruments FT03C force-displacement transducer, connected to a Grass Instruments model 7 D polygraph (see Högestätt *et al.*, 1993). Each segment was initially contracted by a 60 mM K^+ solution (prepared by replacement of 60 mM NaCl with an equimolar amount of KCl) and then by 10 μM phenylephrine (PhE).

Relaxation induced by ACh, A23187 and levcromakalim was studied in vessels contracted by PhE. The PhE concentration was titrated for each vascular segment to give a contraction, amounting to 70–90% of the initial response to 10 μM PhE (Zygmunt *et al.*, 1994a). The vasodilators were then added cumulatively on top of stable contractions. The incubation time with the K-channel inhibitors was 30 min. Preparations were exposed to ouabain, K^+-free Krebs solution or low Cl^- Krebs solution (prepared by replacement of 119 mM NaCl with an equimolar amount of sodium propionate) for 20 min prior to addition of ACh. The volatile anaesthetic, halothane (1 mM) was added twice to the organ bath, i.e. 10 min and immediately before addition of ACh. 1-Heptanol (2 mM) was added 20 min before ACh. Each preparation was exposed to only one treatment. Parallel control experiments with vehicle were performed in the same manner. L-NOARG (0.3 mM) and IM (10 μM) were present in all experiments unless otherwise specified. The incubation period with L-NOARG and IM was at least 40 min.

Calculations and statistics

The negative logarithm of the drug concentration eliciting 50% of the maximal relaxation (pRC_{50}) was determined by linear regression analysis using the values immediately above and below half-maximal response. R_{max} refers to the maximal relaxation achieved (100% denotes a complete reversal of the PhE-induced contraction). Values are presented as mean ± s.e.mean., and n indicates the number of vascular segments (animals) examined. Statistical analysis of pRC_{50} and R_{max} values was by use of Student's t test (two-tailed). Statistical significance was accepted when $P<0.05$.

Drugs

Acetylcholine chloride (Aldrich, Steinheim, Germany); A23187, 4-aminopyridine, N^{ω}-nitro-L-arginine, ouabain octahydrate, (−)-phenylephrine hydrochloride, sodium propionate (propionic acid) and tetrabutylammonium chloride (Sigma, St Louis, MO, U.S.A.); indomethacin (Confortid, Dumex, Copenhagen, Denmark); levcromakalim (SmithKline Beecham, Brentford, U.K.); apamin and dendrotoxin-I (Alomone labs, Jerusalem, Israel); iberiotoxin (Peninsula Inc, Belmont, CA, U.S.A.); native charybdotoxin (Alomone labs, Jerusalem, Israel and Peninsula Inc, Belmont, CA, U.S.A.); synthetic charybdotoxin (Latoxan, Rosans, France); Halothane (ISC Chemicals Ltd, Avonmouth, England). Levcromakalim and A23187 were dissolved in 70% ethanol and methanol, respectively. All other drugs were dissolved in distilled water. Stock solutions were stored at −70°C.

Results

Effects of TBA, apamin, charybdotoxin and iberiotoxin

In the presence of L-NOARG (0.3 mM) and IM (10 μM), ACh and the calcium ionophore A23187 elicited concentration-de-

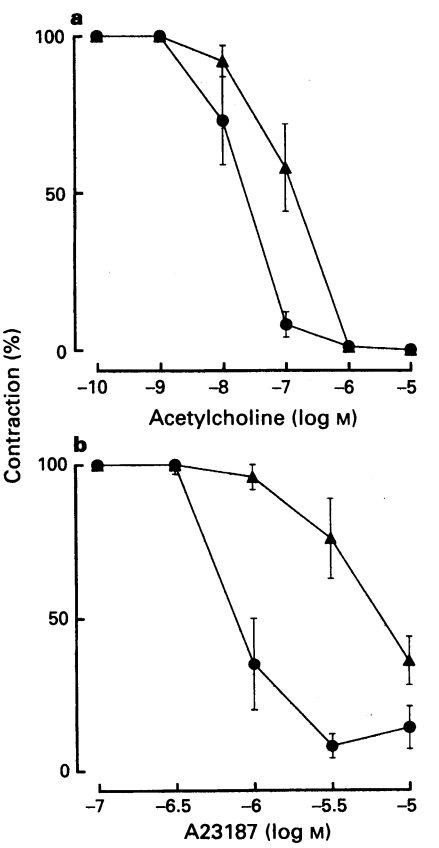

Figure 1 Relaxation induced by ACh (a) or A23187 (b) in the absence (●) and presence of 1 mM TBA (▲) in arteries contracted by PhE. All experiments were performed in the presence of L-NOARG (0.3 mM) and indomethacin (10 μM). Responses are expressed as a percentage of the contraction before addition of ACh or A23187. Data are presented as mean ± s.e.mean of six experiments.

pendent relaxations in the rat hepatic artery (Figure 1). TBA (1 mM) caused a rightward shift of the concentration-response curve for ACh without affecting the maximal relaxation (Figure 1a). The A23187-induced relaxation was also attenuated by TBA (Figure 1b).

Neither apamin nor charybdotoxin, each at a concentration of 0.1 μM (Table 1) or 0.3 μM ($n=4$, data not shown), had an effect on the L-NOARG/IM-resistant relaxation induced by ACh. However, when combined, apamin plus charybdotoxin inhibited (0.1 μM each) or abolished (0.3 μM each) the ACh-induced relaxation (Figure 2a,b). The L-NOARG/IM-resistant relaxation induced by A23187 was also aboblished by apamin (0.3 μM) plus charybdotoxin (0.3 μM; Figure 2c). Iberiotoxin (0.1 μM) alone or in combination with apamin (0.1 μM) did not affect the L-NOARG/IM-resistant relaxation induced by ACh (Table 1). In the absence of L-NOARG, TBA (1 mM) inhibited the ACh-induced relaxation, whereas apamin (0.3 μM) plus charybdotoxin (0.3 μM) had no effect (Figure 3).

The relaxation induced by levcromakalim was abolished by TBA (1 mM, $n=2$, data not shown), but unaffected by apamin (0.3 μM) plus charybdotoxin (0.3 μM). pRC_{50} and R_{max} values for levcrokmakalim were 7.4 ± 0.1 and $99\pm1\%$ in controls, and 7.4 ± 0.1 and $100\pm0\%$ in the presence of the toxin combination, respectively ($n=5$). TBA increased the basal tension in five ($15\pm5\%$ of the initial response to 10 μM PhE) out of 17 vascular segments, whereas the different toxins or toxin combinations had no effect.

Effects of 4-AP and dendrotoxin

4-Aminopyridine (1 mM) had no effect on the maximal ACh-induced relaxation in the presence of L-NOARG and IM, but slightly decreased the sensitivity to ACh (Figure 4, Table 1). When combined with apamin (0.1 μM) and iberiotoxin (0.1 μM), 4-AP did not significantly affect the ACh-induced relaxation (Table 1). Dendrotoxin (0.1 μM) also did not affect the L-NOARG/IM-resistant relaxation induced by ACh (Table 1). At basal tension, addition of 4-AP caused a transient contraction in four ($7\pm2\%$ of the initial response to 10 μM PhE) out of six preparations, whereas dendrotoxin had no effect.

Effect of Cl^- substitution

In low Cl^- Krebs solution, ACh induced a concentration-dependent relaxation in the presence of L-NOARG and IM ($pRC_{50}=7.8\pm0.1$ and $R_{max}=96\pm4\%$, $n=4$). This relaxation did not differ from that obtained in normal Krebs solution ($pRC_{50}=7.5\pm0.0$ and $R_{max}=97\pm1$, $n=4$).

Effects of ouabain and K^+-free Krebs solution

Ouabain (1 mM) did not affect the L-NOARG/IM-resistant relaxation induced by ACh; pRC_{50} and R_{max} values for ACh were 7.6 ± 0.0 and $96\pm1\%$ in controls, and 7.6 ± 0.0 and $95\pm2\%$ in the presence of ouabain, respectively ($n=5$). Readmission of K^+ (4.6 mM KCl) after incubation in K^+-free Krebs solution (20 min) caused a complete relaxation within 1 min (Figure 5). This rapid relaxation was abolished by 1 mM ouabain ($n=3$, Figure 5). Incubation in K^+-free Krebs solution reduced the maximal ACh-induced relaxation by $13\pm6\%$ ($P=0.0463$), whereas the sensitivity to ACh was unaffected; pRC_{50} and R_{max} values for ACh were 7.7 ± 0.2 and $97\pm1\%$ in controls, and 7.5 ± 0.1 and $84\pm6\%$ in K^+-free Krebs solution, respectively ($n=6$).

Effects of halothane and heptanol

The L-NOARG/IM-resistant relaxation induced by ACh was unaffected by 2 mM halothane; pRC_{50} and R_{max} values for ACh were 7.6 ± 0.1 and $96\pm1\%$ in controls, and 7.7 ± 0.2 and $99\pm1\%$ in the presence of halothane, respectively ($n=4$). 1-Heptanol (2 mM) was also unable to affect significantly the ACh-induced relaxation; pRC_{50} and R_{max} values for ACh were 7.6 ± 0.9 and $95\pm1\%$ in controls, and 7.7 ± 0.2 and $80\pm7\%$ in the presence of heptanol, respectively ($n=6$).

Discussion

In principle, membrane hyperpolarization may arise from changes in ion conductances or in the activity of electrogenic transport systems. As shown previously, elevation of the extracellular K^+ concentration abolishes the NO/prostanoid-independent hyperpolarization and relaxation induced by ACh in the rat hepatic artery, suggesting that the responses are caused by either K-channel or Na^+/K^+-pump activation (Zygmunt *et al.*, 1994a,b).

Tetrabutylammonium has been shown to inhibit agonist-induced NO/prostanoid-independent relaxation/vasodilatation in bovine and pig coronary arteries and in rat isolated perfused hearts. It was therefore suggested that calcium-activated K-channels were involved in these responses (Bauersachs *et al.*, 1994; Holzmann *et al.*, 1994). In the present study, TBA caused a five fold rightward shift of the ACh concentration-response curve. The more selective calcium-activated K-channel inhibitors, apamin, charybdotoxin and iberiotoxin had no effect on the L-NOARG/IM-resistant relaxation induced by ACh, whereas a combination of apamin plus charybdotoxin

Table 1 Effects of K-channel inhibitors on the ACh-induced relaxation in arteries contracted by phenylephrine (PhE): L-NOARG (0.3 mM) and indomethacin (10 μM) were present throughout

		Control		*Treatment*	
	n	*pRC_{50}*	*R_{max}*	*pRC_{50}*	*R_{max}*
Tetrabutylammonium 1 mM	6	7.7 ± 0.2	99 ± 1	7.0 ± 0.2[a]	100 ± 0
4-Aminopyridine 1 mM	6	7.8 ± 0.2	100 ± 0	7.4 ± 0.0[b]	100 ± 0
Apamin 0.1 μM	6	7.8 ± 0.2	98 ± 1	7.6 ± 0.1	99 ± 1
Charybdotoxin 0.1 μM	7	7.8 ± 0.2	98 ± 1	7.5 ± 0.2	98 ± 1
Iberiotoxin 0.1 μM	4	7.7 ± 0.2	97 ± 3	8.0 ± 0.2	100 ± 0
Dendrotoxin 0.1 μM	5	7.5 ± 0.1	92 ± 3	7.6 ± 0.2	99 ± 1
Apamin 0.1 μM + iberiotoxin 0.1 μM	5	7.7 ± 0.2	97 ± 2	8.0 ± 0.1	99 ± 1
Apamin 0.1 μM + iberiotoxin 0.1 μM + 4-aminopyridine 1 mM	4	7.5 ± 0.0	92 ± 1	7.5 ± 0.0	100 ± 0

[a]Significantly different from controls ($P<0.05$).
[b]$P=0.0495$ (treatment vs controls).
pRC_{50} denotes the negative logarithm of the drug concentration eliciting 50% of the maximal relaxation.
R_{max} denotes the maximal relaxation achieved (100% denotes a complete reversal of the PhE-induced contraction).
Results are expressed as mean values $\pm$ s.e.mean, and n indicates the number of experiments (animals).

abolished the relaxation. This may indicate that both small and large conductance calcium-activated K-channels are activated in response to ACh, and that activation of either type is sufficient to cause full hyperpolarization and relaxation. However, iberiotoxin, which is considered to be a more selective inhibitor of large conductance calcium-activated K-channels than charybdotoxin, had no effect when combined with apamin. Since charybdotoxin binding sites may exist on a variety of calcium- and/or voltage-sensitive K-channels (see Edwards & Weston, 1994), the effect of charybdotoxin may be attributed to inhibition of a K-channel distinct from large conductance calcium-activated K-channels. The fact that iberiotoxin when combined with apamin had no effect on the L-NOARG/IM-resistant relaxation in the present study does

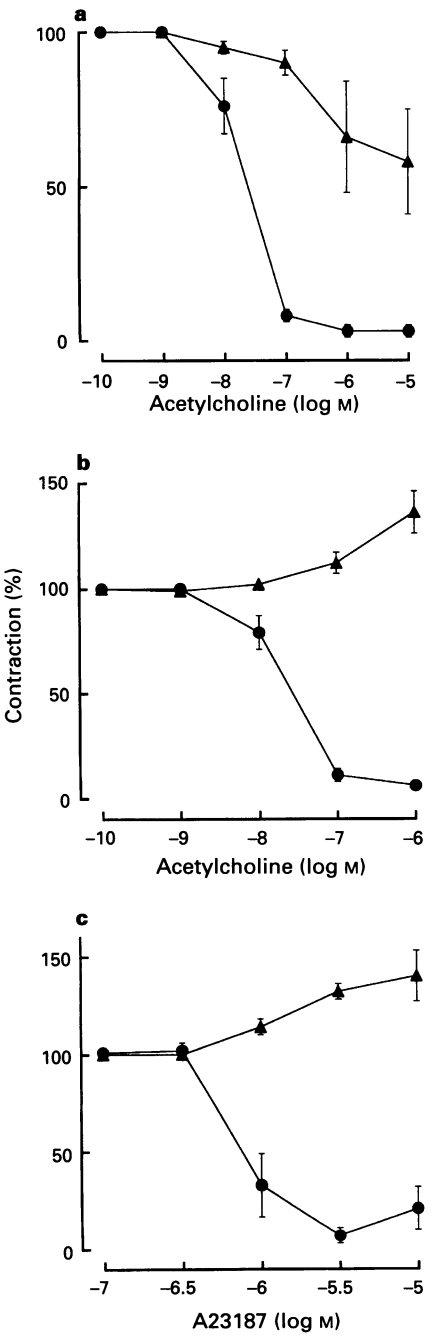

Figure 2 Relaxation induced by ACh (a, b) or A23187 (c) in the absence (●) and presence (▲) of apamin plus charybdotoxin in arteries contracted by PhE. (a) Effect of 0.1 μM apamin plus 0.1 μM charybdotoxin (native). (b) Effect of 0.3 μM apamin plus 0.3 μM charybdotoxin (native $n=5$, synthetic $n=2$). (c) Effect of 0.3 μM apamin plus 0.3 μM charybdotoxin (synthetic). All experiments were performed in the presence of L-NOARG (0.3 mM) and indomethacin (10 μM). Responses are expressed as a percentage of the contraction before addition of ACh or A23187. Data are presented as mean ± s.e.mean of six to seven experiments.

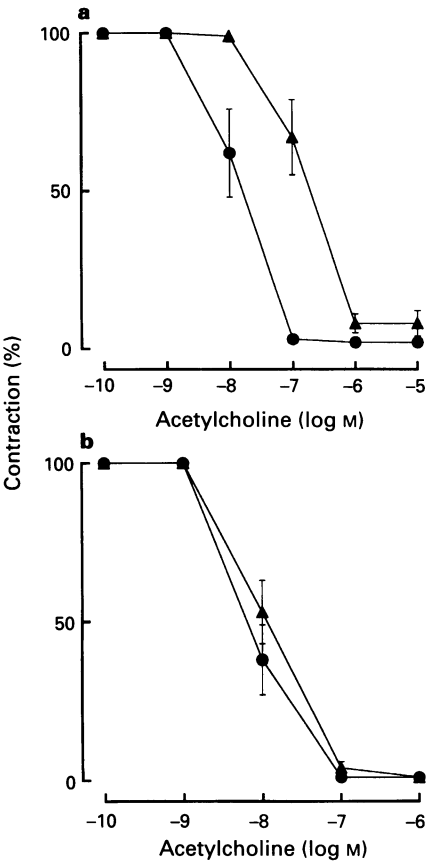

Figure 3 Acetylcholine-induced relaxation in the absence of L-NOARG with (●) and without (▲) 1 mM TBA (a) and 0.3 μM apamin plus 0.3 μM synthetic charybdotoxin (b) in arteries contracted by PhE. Indomethacin (10 μM) was present in all experiments. Responses are expressed as a percentage of the contraction before addition of ACh. Data are presented as mean ± s.e.mean of six experiments.

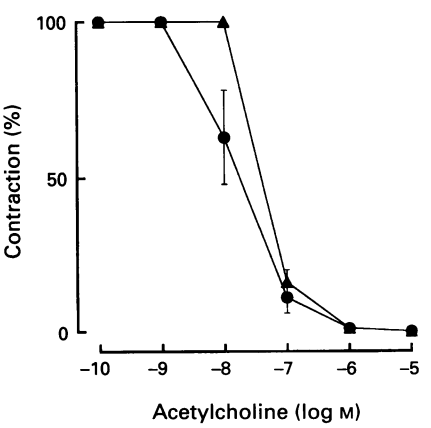

Figure 4 Relaxation induced by ACh in the absence (●) or presence (▲) of 1 mM 4-AP in arteries contracted by PhE. All experiments were performed in the presence of L-NOARG (0.3 mM) and indomethacin (10 μM). Responses are expressed as a percentage of the contraction before addition of ACh. Data are presented as mean ± s.e.mean of six experiments.

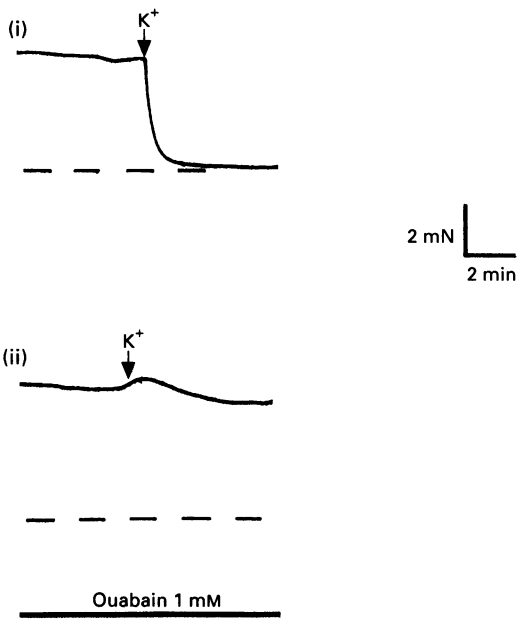

Figure 5 Relaxation induced by K^+ readmission (4.6 mM KCl) after incubation in K^+-free Krebs solution in arteries contracted by PhE in the absence (i) and presence (ii) of 1 mM ouabain. All experiments were performed in the presence of L-NOARG (0.3 mM) and indomethacin (10 μM). Broken lines indicate baseline tension before addition of PhE.

not exclude a contribution of large conductance calcium-activated K-channels, but may rather indicate that, in addition to small and large conductance calcium-activated K-channels, another charybdotoxin-sensitive K-channel(s) is involved in the response. Alternatively, apamin and charybdotoxin may interact with a single type of K-channel that requires binding of both toxins for inhibition to occur. However, voltage-sensitive K-channels do not seem to be of importance for the response, since (i) 4-AP had only a small effect on the L-NOARG/IM-resistant relaxation, and (ii) dendrotoxin, which is a more potent and selective inhibitor of voltage-sensitive K-channels (see Edwards & Weston, 1994) and (iii) a triple combination of 4-AP plus apamin plus iberiotoxin was unable to affect the response significantly.

It may be argued that TBA and apamin plus charybdotoxin prevented the endothelium-dependent smooth muscle hyperpolarization and relaxation by interacting with muscarinic receptors and/or K-channels on endothelial cells. However, with regard to the toxin combination, this seems unlikely, since (i) the L-NOARG/IM-resistant relaxation induced by the receptor-independent activator A23187 was abolished and (ii) the ACh-induced relaxation in the absence of L-NOARG was unaffected by apamin plus charybdotoxin. This indicates not only that the toxin combination had no effect on endothelial ACh receptors, but also that the ability of the endothelial cells to release NO was intact in the presence of the toxins. Provided that ACh-induced hyperpolarization and NO release share a common activation mechanism, it seems reasonable to assume that the release of a hypothetical endothelium-derived hyperpolarizing factor (EDHF) was intact in the presence of the toxins. In support of a common activation mechanism, an increase of the intracellular calcium concentration in the endothelial cells appears to be an important stimulus not only for the formation of NO (Busse *et al.*, 1993), but also for endothelium-dependent hyperpolarization (Suzuki & Chen, 1990; Nagao & Vanhoutte, 1993). This seems to be true also in the rat hepatic artery, since A23187 elicits an endothelium-dependent relaxation in both the absence (Zygmunt *et al.*, 1995) and presence (present study) of L-NOARG and IM. It also seems unlikely that the effect of charybdotoxin was due to impurities (see Edwards & Weston, 1994), since native charybdotoxin purchased from two different suppliers and synthetic charybdotoxin were equally effective. A direct inhibitory effect of TBA on the endothelium has been demonstrated previously (Groschner *et al.*, 1992) and cannot be excluded in the present study, since the ACh-induced relaxation in the absence of L-NOARG was inhibited by TBA. Clearly, the action of TBA cannot be explained simply by an action on endothelial muscarinic receptors and/or K-channels, since the substance also inhibited the L-NOARG/IM-resistant relaxation induced by A23187. Moreover, TBA abolished the levcromakalim-induced relaxation, adding further complexity to the action of this compound.

In a previous study, glibenclamide had no effect on the L-NOARG/IM-resistant hyperpolarization and relaxation induced by ACh in the rat hepatic artery, whereas the corresponding reponses to levcromakalim were abolished (Zygmunt *et al.*, 1994b). This led us to conclude that ATP-sensitive K-channels do not mediate these responses. However, as shown by Edwards *et al.* (1995), ATP-sensitive K-channels can be activated in the presence of glibenclamide. Thus, glibenclamide may be an unreliable pharmacological tool to investigate whether endothelium-dependent hyperpolarization is mediated by ATP-sensitive K-channels. In the present study, the levcromakalim-induced relaxation was unaffected by apamin plus charybdotoxin, which abolished the L-NOARG/IM-resistant relaxation induced by ACh. This finding supports our previous conclusion regarding the role of ATP-sensitive K-channels in endothelium-dependent hyperpolarization and relaxation in the rat hepatic artery.

Activation of chloride channels may lead to a depolarization or hyperpolarization depending on the prevailing membrane and chloride equilibrium potential. Replacement of NaCl with sodium propionate did not affect the L-NOARG/IM-resistant relaxation induced by ACh in the present study, refuting the idea that chloride channels participated in the response. The ACh-induced relaxation was also unaffected by ouabain, whereas the rapid relaxation elicited by readmission of K^+ after incubation in K^+-free Krebs solution was abolished. Since K^+-induced relaxation may be used as a functional indicator of Na^+/K^+-ATPase activity in vascular smooth muscle (Webb & Bohr, 1978; Feletou & Vanhoutte, 1988), the results indicate that Na^+/K^+-ATPase was indeed inhibited by ouabain in the present study. Therefore, the results do not support the idea that endothelium-dependent hyperpolarization is caused by stimulation of Na^+/K^+-ATPase in the rat hepatic artery. The observation that the ACh-induced relaxation was attenuated in K^+-free Krebs solution may not be related to inhibition of Na^+/K^+-ATPase, since reduction of the external K^+ concentration may directly affect K-channel function (Capoid & Ogden, 1989; Adeagbo & Malik, 1990; McCarron & Halpern, 1990).

The gap junction uncoupler, halothane (Bennett *et al.*, 1991; Watts *et al.*, 1994) had no effect on the L-NOARG/IM-resistant relaxation in the rat hepatic artery. Although halothane was added to the tissue bath immediately before ACh, the possibility cannot be excluded that the lack of effect was due to the fact that halothane is volatile. In porcine coronary arteries, continuous superfusion with halothane (1.74 mM) inhibited the conduction of the isoprenaline-induced smooth muscle hyperpolarization to the endothelial cells, whereas the endothelium-dependent smooth muscle hyperpolarization induced by bradykinin was unaffected (Bény & Pacicca, 1994). Thus, although myoendothelial gap junctions may exist in porcine coronary arteries, they are not obligatory for the endothelium-dependent hyperpolarization to occur (Bény & Pacicca, 1994). In another study on porcine coronary arteries, it was suggested that the L-NOARG/IM-resistant relaxation induced by bradykinin requires the presence of functional myoendothelial gap junctions, since the response was inhibited by 1-heptanol (Kühberger *et al.*, 1994). In the rat hepatic artery, treatment with 1-heptanol had no effect on the L-NOARG/IM-resistant relaxation induced by ACh. Thus,

myoendothelial gap junctions do not seem to be of importance for endothelium-dependent smooth muscle hyperpolarization and relaxation in this artery. However, further studies are required to establish whether the ACh-induced relaxation in the rat hepatic artery is mediated by a chemical factor.

In conclusion, the results suggest that the L-NOARG/IM-resistant relaxation induced by ACh in the rat hepatic artery is mediated by activation of K-channels sensitive to TBA and a combination of apamin plus charybdotoxin. Chloride channels, Na^+/K^+-ATPase and gap junctions do not appear to play a major role in the response. It is proposed that endothelial cell activation induces secretion of an endothelium-derived hyperpolarizing factor(s) (EDHF), distinct from NO and cyclo-oxygenase products, which activates more than one type of K-channel on the smooth muscle cells. Alternatively, a single type of K-channel, to which both apamin and charybdotoxin must bind for inhibition to occur, may be the target for EDHF.

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