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Differential effects of acetylcholine, nitric oxide and levcromakalim on smooth muscle membrane potential and tone in the rabbit basilar artery

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1 Endothelium-dependent hyperpolarization of smooth muscle cells in isolated, pre-contracted segments of rabbit basilar artery in response to acetycholine (100 μM) was abolished in the presence of glibenclamide (10 μM).

2 Acetylcholine-evoked relaxation was unaffected by either glibenclamide or 65 mM potassium chloride, indicating that the change in membrane potential did not form an essential component of relaxation and that high concentrations of potassium did not inhibit the release or action of endothelium-derived relaxing factor in this vessel.

3 Saturated solutions of nitric oxide (NO) gas in solution (150 μM), which evoked maximal relaxation of arterial segments pre-contracted and depolarized by noradrenaline (10–100 μM), did not alter the membrane potential of either unstimulated or depolarized smooth muscle cells.

4 The potassium channel opener levcromakalim, evoked concentration-dependent relaxation and hyperpolarization in pre-constricted smooth muscle cells. The threshold concentrations for hyperpolarization and relaxation, the EC_{50} values and the maximally effective concentration of levcromakalim (around 30 nM, 150 nM and 10 μM, respectively) were not significantly different, and both components of the response were inhibited by glibenclamide (10 μM), indicating a close coupling between the two responses.

5 In the presence of 65 mM potassium chloride, the hyperpolarization to levcromakalim was abolished, while a small relaxation (25 ± 4%) persisted, indicating an additional mechanism for relaxation to this agent.

6 These results show that different mechanisms underlie the relaxant action of potassium channel openers, NO and endothelium-derived factors in cerebral arteries and provide further evidence that in the basilar artery, in contrast to some other vessels, endothelium-dependent hyperpolarization to acetylcholine is not important for smooth muscle relaxation.

Keywords: Glibenclamide; hyperpolarization; levcromakalim; membrane potential; nitric oxide; potassium channels; vascular smooth muscle

Introduction

The relaxation of blood vessels evoked by muscarinic agonists is usually accompanied by hyperpolarization of the smooth muscle cell membrane, both events being mediated by the release of a diffusible factor from the endothelium (Chen *et al.*, 1988; 1991; Feletou & Vanhoutte, 1988). Endothelium-derived relaxing factor (EDRF) has now been identified as nitric oxide (NO; Palmer *et al.*, 1987), or a closely related compound, but the possibility that NO may contribute to endothelium-dependent hyperpolarization and the mechanism underlying this response is the subject of some controversy.

Inhibitors of NO synthase did not reduce acetylcholine-evoked hyperpolarization in the guinea-pig coronary and rat small mesenteric artery, indicating the involvement of an endothelium-derived hyperpolarizing factor (EDHF) distinct from NO (Chen *et al.*, 1991; Garland & McPherson, 1992). However, in the guinea-pig uterine artery both the relaxation and hyperpolarization evoked by acetylcholine were depressed by the NO synthase inhibitor L-N^G-monomethyl arginine (L-NMMA), indicating that NO may contribute to both smooth muscle hyperpolarization and relaxation in this particular vessel (Tare *et al.*, 1990). Additionally, in both the guinea-pig uterine artery and the rat small mesenteric artery, exogenous NO can stimulate both smooth muscle relaxation and membrane hyperpolarization under certain conditions (Tare *et al.*, 1990; Garland & McPherson, 1992).

The role of glibenclamide-sensitive potassium channels in the endothelium-dependent hyperpolarization evoked by acetylcholine is also controversial and may vary between different vessels. For example, in the rabbit middle cerebral artery, acetylcholine-evoked hyperpolarization was reduced by the sulphonylurea compound glibenclamide, whereas in the guinea-pig isolated coronary artery and rat small mesenteric arteries this agent did not block responses to acetylcholine (Standen *et al.*, 1989; Brayden, 1990; Eckman *et al.*, 1992; Garland & McPherson, 1992). Furthermore, in the rat small mesenteric artery, although acetylcholine-induced hyperpolarization was not abolished by glibenclamide, hyperpolarization to NO was inhibited (Garland & McPherson, 1992), providing further indirect evidence that NO may contribute to endothelium-dependent hyperpolarization in some vessels.

Glibenclamide also inhibits the smooth muscle relaxation which is evoked by potassium channel opening drugs (KCOs) such as levcromakalim, suggesting that membrane hyperpolarization, mediated by the opening of glibenclamide-sensitive potassium channels, is the major mechanism underlying the decrease in vascular tone (Buckingham *et al.*, 1989; Winquist *et al.*, 1989; McHarg *et al.*, 1990). This hypothesis is based largely on separate experiments showing that KCOs can increase the resting membrane potential of unstimulated vascular smooth muscle cells, and can also relax isolated, pre-contracted vessels. There is relatively little information on the membrane effects of KCOs under conditions of smooth muscle depolarization. Minoxidil sulphate and cromakalim have both been shown to reduce the subsequent depolarizing action of noradrenaline in the rabbit portal vein and

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mesenteric artery, respectively (Leblanc *et al.*, 1989; McHarg *et al.*, 1990). However, although smooth muscle relaxation elicited by KCO's is reduced in isolated arteries precontracted with high concentration of extracellular potassium, consistent with a role for potassium channels in relaxation (Parsons *et al.*, 1991a), in only one study have the membrane events underlying this response been reported (Nakashima *et al.*, 1990).

The fact glibenclamide can inhibit the membrane hyperpolarization evoked by NO, and in some vessels that to endothelium derived factor(s), suggests an important role for the potassium channels which are activated by agents like levcromakalim, which are also glibenclamide-sensitive. The reported variation in the ability of NO to elicit smooth muscle hyperpolarization, and in the sensitivity of endothelium-dependent hyperpolarization to glibenclamide, could then be explained in a number of ways, including a variation in either the endothelium-derived mediator of hyperpolarization or in the distribution of potassium channels. Regional differences in the distribution of potassium channels have been suggested within the cerebral circulation of the rat, as not all of the arteries arising from the circle of Willis are hyperpolarized by KCOs (McCarron *et al.*, 1991; McPherson & Stork, 1992).

In the rabbit isolated basilar artery, acetylcholine-evoked hyperpolarization is both small in amplitude and transient. In addition, it is diminished on repeated application of the agonist. In contrast, the relaxation to acetylcholine is both sustained and reproducible with repeated exposures (Rand & Garland, 1992). However, both responses are attenuated by inhibitors of NO synthase indicating that, as in the guinea-pig uterine artery, NO may contribute to both components of the response to acetylcholine (Rand & Garland, 1992). Surprisingly, exogenous NO failed to evoke any significant hyperpolarization in smooth muscle cells of the rabbit basilar artery, even at concentrations which caused a maximal reversal of induced tone (15 μM; Rand & Garland, 1992). However, this concentration was close to the threshold for membrane hyperpolarization to NO in the rat mesenteric artery, so that higher concentrations might be required to demonstrate hyperpolarization if the potassium channels which mediate this response are sparse in this particular vessel (Garland & McPherson, 1992).

The aims of the present study were to investigate the glibenclamide-sensitivity of acetylcholine-evoked hyperpolarization and relaxation in the rabbit basilar artery, and to examine the possibility that very high concentrations of NO may be required for smooth muscle hyperpolarization. Furthermore, the effects of the KCO, levcromakalim, on both smooth muscle tone and membrane potential, under both resting and depolarized conditions, were also examined. The aim was to determine if the small size of the membrane responses evoked by acetylcholine, and the apparent lack of hyperpolarization to NO, could reflect an absence of KCO and glibenclamide-sensitive potassium channels in this artery.

Methods

White rabbits of either sex (2–3 kg) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.v.) and killed by rapid exsanguination. The brain was removed and placed in Krebs buffer at room temperature. The basilar artery was carefully removed, cleaned and cut into cylindrical segments 2 mm in length. Segments were then mounted in a tissue chamber for simultaneous recording of changes in smooth muscle membrane potential and tension, as previously described (Garland, 1987). Briefly, two tungsten wires (40 μm diameter) were passed through the lumen of the segment and each wire attached to a metal foot in a myograph (model 400 A, J.P. Trading, Denmark). The tissue segment was stretched between the wires under a previously determined optimal preload of 500 mg and superfused at 5–6 ml min^{-1} with Krebs buffer which had been bubbled with 95% O_2/5% CO_2. All experiments were carried out on tissues with a functionally intact endothelium unless otherwise stated.

Drugs were equilibrated with the perfusate before it entered the tissue chamber. Nitric oxide solutions were injected close to the artery segment, from a gas-tight syringe in volumes not greater than 200 μl.

Electrophysiology

Measurement of smooth muscle membrane potential was made with a glass microelectrode advanced through the adventitial surface of the artery segment. The electodes were filled with 2 M KCl and had resistances of 60–120 MΩ. Membrane electrical events were recorded through a high-input impedance d.c. preamplifier (Neurolog 102G) and, together with data from the isometric force transducer, stored on disc (CVMS, McPherson Scientific).

Solutions and drugs

Tissues were maintained in Krebs buffer of the following composition (mM): NaCl 122, $NaHCO_3$ 25.5, KCl 5.2, $MgSO_4$ 1.2, $CaCl_2$ 1.6, disodium EDTA 0.027, ascorbate 0.114 and glucose 9.4. All K^+-rich Krebs solutions were prepared by direct replacement of NaCl with KCl. At the end of each experiment, tissues were maximally contracted with 100 mM K^+-Krebs solution.

Drugs used were acetylcholine chloride (BDH), noradrenaline bitartrate (arterenol, Sigma), levcromakalim (gift from Smith Kline Beecham) and glibenclamide (gift from Hoechst).

Preparation of nitric oxide solutions

Nitric oxide gas (research grade, BDH) was injected into Krebs solution which had been bubbled with helium (BOC) for 45–60 min. Nitric oxide solution was injected into the tissue chamber in volumes of 200 μl with a gas tight syringe. Control injections of helium-gassed Krebs solution were made to assess the extent of any potential injection artifacts.

Analysis of data

Relaxations are expressed as a percentage decrease in the tone induced by either noradrenaline or potassium chloride. Data are expressed as mean ± s.e.mean. The significance between mean values was calculated by Student's t test, with rejection of the null hypothesis at the 5% level ($P<0.05$).

Results

Membrane and tension responses to acetylcholine

Smooth muscle cells in the basilar artery were electrically quiescent, and the mean resting membrane potential was -63.7 ± 7.1 mV (80 cells from 28 preparations). When first applied to noradenaline precontracted tissues (mean background contraction and depolarization of 4.5 ± 0.9 mN and 10.5 ± 2.2 mV, $n=4$), acetylcholine (100 μM) evoked 95 ± 3.5% relaxation of the induced tone and hyperpolarized the smooth muscle cell membrane by 8.1 ± 1.0 mV ($n=4$ paired observations). Subsequent exposures to acetylcholine were followed by a relaxation of similar magnitude (93.9 ± 4.6% on fifth exposure). However, as previously described (Rand & Garland, 1992), the amplitude of the accompanying hyperpolarization decreased with each application of acetylcholine, although in contrast to the previous study, a small but significant change in membrane potential was still observed even on a fifth exposure (4.1 ± 1.5 mV; $n=4$). The threshold concentrations for acetylcholine-evoked hyperpolarization and relaxation were 1 μM and 0.1 μM, respectively (Figure 1a).

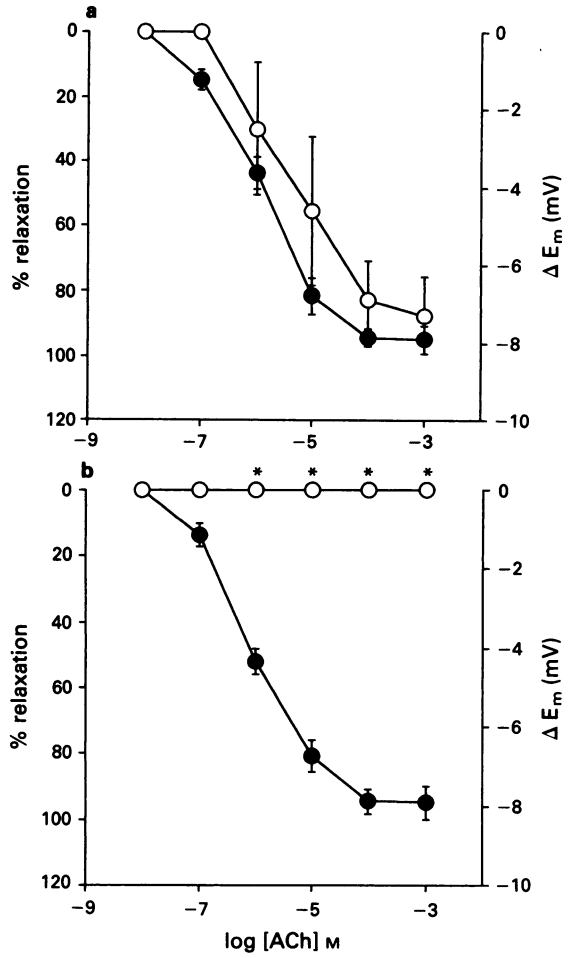

Figure 1 Mean concentration-response curves for acetylcholine in the rabbit basilar artery precontracted with noradrenaline (10–100 μM). Points show relaxation (●) and hyperpolarization (○) and are the mean ± s.e.mean from 4 separate experiments. (a) Control concentration-response curves for the second application of acetylcholine. (b) Concentration-response curves in the presence of glibenclamide. $*P<0.05$ compared to control responses.

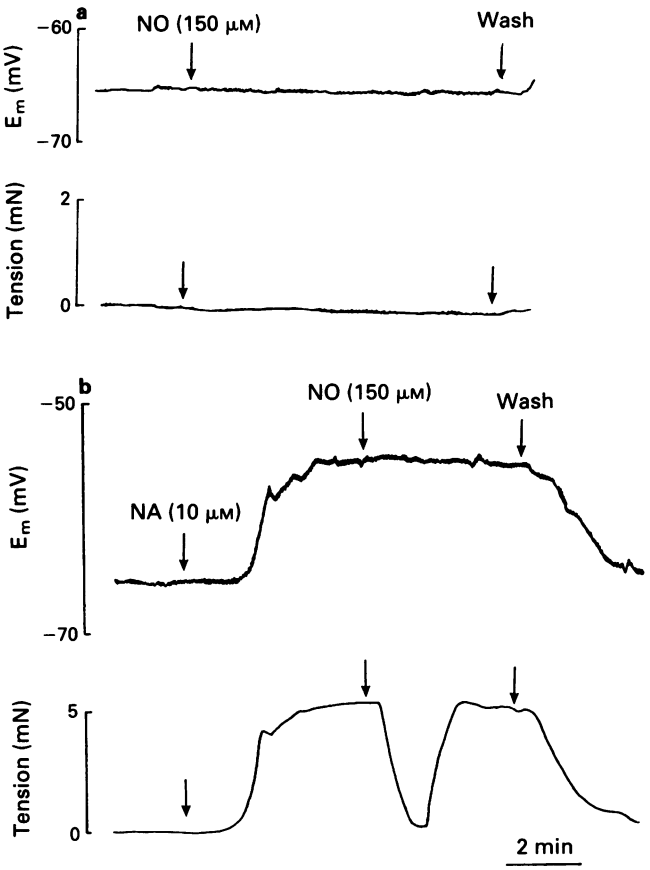

Figure 2 Representative traces showing simultaneous records of membrane potential and changes in tension elicited by NO in the absence and presence of noradrenaline to induce contraction and depolarization. (a) NO (150 μM) had no effect on resting membrane potential or tension in the unstimulated rabbit basilar artery. The resting membrane potential of the cell shown in this trace was − 67 mV. (b) Noradrenaline (10 μM) elicited contraction of approximately 5 mN and depolarized the smooth muscle by approximately 10 mV (resting membrane potential − 65 mV). NO (150 μM) initiated a transient relaxation of the induced tone but had no effect on the membrane potential of the smooth muscle cell.

When glibenclamide (10 μM) was added after the first exposure to acetylcholine, the subsequent membrane hyperpolarization was abolished, although at this time relaxations were not significantly altered (Figures 1b). Furthermore, the acetylcholine-evoked relaxations were also unaltered when potassium chloride (65 mM) was used to induce tone instead of noradrenaline. The EC_{50} values for acetylcholine-evoked relaxation in the presence of either noradrenaline or potassium were 1.5 ± 0.2 μM and 1.65 ± 0.4 μM, respectively ($n = 4$; $P>0.05$), and the maximal relaxations obtained were 94.8 ± 3.8% and 94.5 ± 4.5%, respectively $n = 4$; $P>0.05$).

Membrane and tension responses to nitric oxide

In tissues contracted and depolarized with noradrenaline (10–100 μM), NO (0.5–15 μM) initiated transient relaxations which reversed within 20–25 s. Maximal relaxation of induced tone (85.4 ± 6.7%; $n = 4$) was achieved at a concentration of 15 μM NO and the EC_{50} value was 2.0 ± 0.9 μM ($n = 4$).

Over the concentration-range which produced relaxation of induced tone, NO (0.5–15 μM) had no significant effect on the membrane potential of unstimulated smooth muscle cells, in contrast to the small hyperpolarization (around 2 mV) reported by Rand & Garland (1992). Furthermore, at a concentration 10 times higher than that required to evoke maximal relaxation, 150 μM, NO did not elicit any smooth muscle hyperpolarization, either in the absence (Figure 2a) or presence (Figure 2b) of prior membrane depolarization to noradrenaline.

Membrane and tension responses to levcromakalim

Levcromakalim (1–100 μM) evoked concentration-dependent hyperpolarization in unstimulated smooth muscle cells (mean resting membrane potential − 63.3 ± 6.8 mV; 39 cells from 14 preparations), which was maintained for over 20 min in the continued presence of the drug. In these experiments, levcromakalim had no significant effect on the resting level of tone. The maximal hyperpolarization to levcromakalim (100 μM) was 14 ± 3.2 mV ($n = 6$). Glibenclamide (10 μM), had no significant effect on either the tone or membrane potential of unstimulated smooth muscle cells during exposures of up to 20 min, but abolished levcromakalim-evoked hyperpolarization.

In tissues depolarized and contracted with noradrenaline (mean background contraction and depolarization 4.98 ± 0.7 mN and 12.7 ± 2.5 mV, respectively; $n = 4$), levcromakalim (0.01–10 μM) evoked concentration-dependent relaxation of the induced tone, which was accompanied by smooth muscle hyperpolarization. The threshold concentration (30 nM), EC_{50} values (140 ± 25 nM and 175 ± 15 nM; $n = 4$) and the concentration of levcromakalim required for a maximal response (10 μM; $n = 4$) with both hyperpolarization and relaxation were not significantly different (Figure 3a). Following prior exposure to glibenclamide (10 μM) for

Table 1 Comparison of relaxation and smooth muscle hyperpolarization evoked by levcromakalim in the rabbit basilar artery pre-contracted with noradrenaline or potassium chloride

	Noradrenaline (10–100 μM)	25 mM *KCl*	35 mM *KCl*	65 mM *KCl*
Mean level of contraction (mN)	4.98 ± 0.7	3.1 ± 0.6	14.5 ± 2.1	23.0 ± 3.9
Mean level of depolarization (mV)	12.7 ± 2.5	22.1 ± 2.9	29.4 ± 2.0	44.1 ± 4.0
% maximal relaxation to levcromakalim (100 μM)	95.0 ± 5.1	97.0 ± 4.1	74.0 ± 6.9	25.0 ± 4.0
Maximal membrane potential change to levcromakalim (100 μM; mV)	25.0 ± 2.3	26.0 ± 2.1	15.0 ± 4.9	0

Each value is the mean of 4 observations ± s.e.mean.

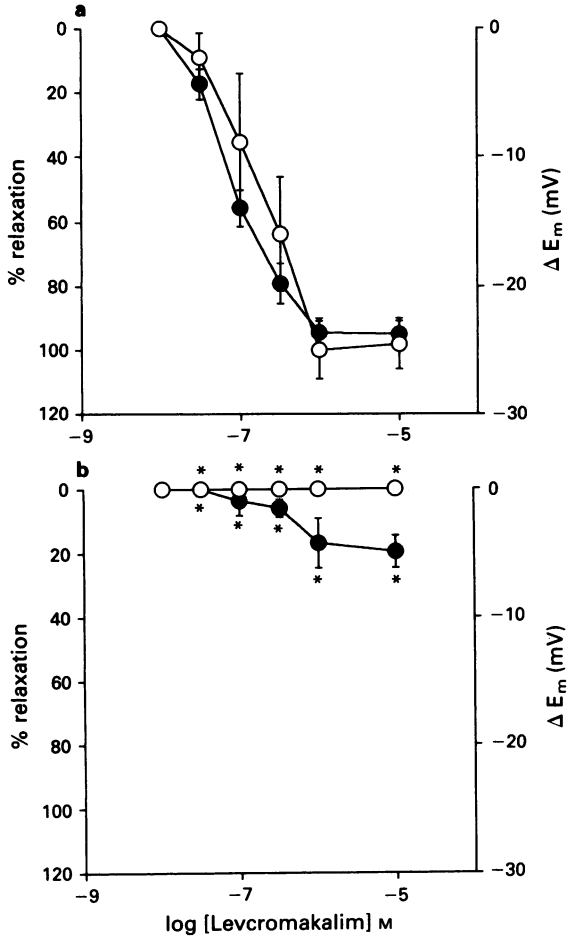

Figure 3 Mean concentration-response curves for levcromakalim in the rabbit basilar artery contracted with noradrenaline (10–100 μM). Points show relaxation (●) and hyperpolarization (○) and are the mean ± s.e.mean from 4 separate experiments. (a) Control concentration-response curves to levcromakalim. (b) Concentration-response curves in the presence of glibenclamide (10 μM). *$P<0.05$ compared to controls.

20 min, the maximal levcromakalim-evoked relaxation was reduced to 19.4 ± 5.0% ($n = 4$) and the accompanying smooth muscle hyperpolarization was abolished (Figure 3b). Levcromakalim-evoked responses were also reduced in the presence of elevated external potassium concentrations. In arterial segments preconstricted with 25 mM potassium chloride, levcromakalim evoked concentration-dependent relaxation and hyperpolarization which was not significantly different from the responses observed in noradrenaline precontracted tissues. The maximal changes in tension and smooth muscle membrane potential evoked by levcromakalim in the presence of 25 mM potassium chloride were 97 ± 4.1% and 26 ± 2.1 mV respectively ($n = 4$; $P>0.05$). In contrast, when 35 mM and 65 mM potassium chloride were used, both the smooth muscle relaxation and hyperpolarization evoked by levcromakalim were depressed. Table 1 shows the maximal relaxation and change in membrane potential evoked by levcromakalim (100 μM) in tissues precontracted with either noradrenaline or potassium chloride. In the presence of 35 mM potassium chloride, the maximal relaxation and change in membrane potential evoked by levcromakalim were reduced by approximately 24% and 42%, respectively. In arterial segments contracted with 65 mM potassium, levcromakalim-evoked hyperpolarization was abolished although a small relaxation (25 ± 4%) still persisted.

Discussion

These data confirm and extend our previous study, which indicated that membrane hyperpolarization did not make an important contribution to the smooth muscle relaxation evoked by either acetylcholine or exogenous NO in the rabbit basilar artery (Rand & Garland, 1992). The present study demonstrated that the small hyperpolarization evoked by acetylcholine was inhibited by glibenclamide, whereas smooth muscle relaxation was unaffected. The ability of glibenclamide to block acetylcholine-induced hyperpolarization is similar to its action in the rabbit middle cerebral artery (Standen *et al.*, 1989; Brayden, 1990). The maximal hyperpolarization in response to acetylcholine, at 8 mV, was not marked, but by comparison to the action of levcromakalim might be predicted to stimulate a relaxation of around 30–40% (see Figure 3). This relaxation would, however, be masked by the relaxation induced by other mechanisms initiated by lower concentrations of acetycholine. As the activation of glibenclamide-sensitive potassium channels also mediates smooth muscle hyperpolarization to the KCO levcromakalim and, in some vessels, NO and endothelium-derived factors, these agents may all share an ability to activate the same type of potassium channel under certain conditions.

Smooth muscle relaxation in the basilar artery was also unaffected by precontraction with a high concentration of potassium (65 mM), a concentration which is known to inhibit smooth muscle hyperpolarization to acetylcholine (Chen *et al.*, 1989; Waldron *et al.*, 1993), demonstrating that this concentration of potassium does not inhibit either the action or the release of EDRF in this vessel.

Glibenclamide did not modify either the resting membrane potential or tension in the basilar artery, indicating a low open probability for glibenclamide-sensitive channels at this potential. This observation was not complicated by an action exerted via the endothelium, as glibenclamide was without effect in both endothelium-intact and denuded tissues. Glibenclamide also had no effect on resting tension and membrane potential in the rabbit middle cerebral artery (Brayden, 1990), whereas in the rat small mesenteric and guinea-pig coronary artery, glibenclamide caused membrane depolarization and smooth muscle contraction in unstimulated tissues (McPherson & Angus, 1990; 1991; Eckman *et al.*, 1992). These contrasting results suggest a variation in the characteristics of the glibenclamide-sensitive potassium channels present in these different vessels and in their contribution

to smooth muscle tone. Variation has also been observed in the sensitivity of acetycholine-evoked hyperpolarization to glibenclamide. The mesenteric artery smooth muscle cells, although depolarized by glibenclamide, developed a hyperpolarization which was not reduced in overall size by glibenclamide (Garland & McPherson, 1992), while in the basilar artery the relatively small hyperpolarization was abolished. These differences could be explained by different populations of potassium channels in the two arteries.

The majority of evidence, from a range of isolated blood vessels, suggests that the endothelium-dependent hyperpolarization which is evoked by cholinomimetics is not mediated by NO. For example, in the guinea-pig coronary artery, endothelium-dependent relaxation can be reduced independently of hyperpolarization by the NO synthase inhibitor nitroarginine. In contrast, a number of studies including the present one, have failed to demonstrate significant membrane hyperpolarization in response to concentrations of exogenous NO which are capable of stimulating maximal smooth muscle relaxation (Komori *et al.*, 1988; Brayden, 1990; Chen *et al.*, 1991; Rand & Garland, 1992). However, NO-evoked hyperpolarization has been demonstrated in smooth muscle cells in rat small mesenteric arteries and in the guinea-pig uterine artery, indicating that in some vessels NO may at least contribute to acetylcholine-induced hyperpolarization (Tare *et al.*, 1990; Garland & McPherson, 1992). In the rabbit isolated basilar artery, although NO did not alter the membrane potential, both the hyperpolarization and relaxation stimulated by acetylcholine were reduced by the NO synthase inhibitors L-NMMA and N^G-nitro-L-arginine methyl ester (L-NAME), indicating that NO contributes in some way to the hyperpolarization in this artery (Rand & Garland, 1992). Why NO failed to alter the membrane potential in the basilar artery when glibenclamide-sensitive potassium channels are present, and NO can activate such channels in other arteries, is not clear. One possibility is that glibenclamide can block more than one type of potassium channel, fitting in with its variable action on the resting membrane potential in different arteries. In this case, the NO-sensitive channels present in the mesenteric artery may not be present in the basilar artery. In the basilar artery, even saturated solutions of NO failed to change the smooth muscle membrane potential although they caused a total reversal of contraction. These concentrations of NO were 10 fold greater than in our previous study, when we did observe a slight hyperpolarization of 2 mV, which was not large enough to contribute significantly to relaxation. The reason for our failure to record similar effects in the present study is not clear, particularly as the sensitivity of the tissues to NO and the maximal relaxation attained were similar in both studies, and the degree of preconstriction and depolarization to noradrenaline were comparable.

In contrast to NO, levcromakalim evoked both concentration-dependent smooth muscle relaxation and hyperpolarization in the rabbit basilar artery, indicating that the lack of hyperpolarization to NO did not reflect an absence of levcromakalim-sensitive potassium channels. The different responses to levcromakalim and NO in the basilar artery are in contrast to studies in the rat mesenteric artery (McPherson & Angus, 1991; Garland & McPherson, 1992), where both cromakalim and NO hyperpolarized the resting membrane potential, both via a glibenclamide-sensitive pathway. Taken together, these observations again suggest a variation in the type of potassium channels which are present in each of these arteries.

Although membrane hyperpolarization to NO has yet to be investigated at the single channel level, the picture is much clearer in the case of KCOs, based on extensive patch-clamp studies with levcromakalim (Noack *et al.*, 1992a,b). These data have provided evidence that levcromakalim can activate small conductance potassium channels, which are ATP-sensitive and appear to be identical to the channels which are opened by depletion of the cellular substrates required for oxidative metabolism. These experiments also showed that levcromakalim could influence the open probability of other potassium channels, such as those which carry the delayed rectifier current. Levcromakalim and cromakalim have very similar actions on the mesenteric and basilar arteries, so may very well act by similar mechanisms in these vessels. In contrast, the hyperpolarization to NO, but not cromakalim was blocked by prior depolarization in the mesenteric artery, consistent with the idea of separate potassium channels mediating the responses to KCOs and NO, with both sensitive to glibenclamide.

Unlike NO, levcromakalim does not stimulate guanylyl cyclase in vascular smooth muscle cells, rather the relaxation appears to follow the opening of membrane potassium channels, subsequent hyperpolarization and a reduction in the open probability of voltage-dependent calcium channels (Hamilton *et al.*, 1986; Weir & Weston, 1986; Coldwell & Howlett, 1987; Taylor *et al.*, 1988; Noack *et al.*, 1992a,b). Most data on KCO's have been derived from separate measurements of smooth muscle tension and membrane potential, giving a circumstantial link between relaxation and hyperpolarization (Cavero *et al.*, 1989; Winquist *et al.*, 1989). Additionally, in vessels such as the guinea-pig coronary artery, relaxation of noradrenaline-contracted arterial segments by levcromakalim, was depressed by the presence of glibenclamide, indicating that the two events are causally linked (Eckman *et al.*, 1992). To date, there have been only a limited number of studies on the membrane events which accompany smooth muscle relaxation in response to KCOs in depolarized tissues. In the present study, simultaneous recordings showed that levcromakalim stimulated concentration-dependent hyperpolarization in unstimulated segments of the rabbit basilar artery and reversed the depolarization and contraction in the presence of noradrenaline. Both components of the response were inhibited by pre-incubation with glibenclamide (10 μM). Also, in the presence of 65 mM potassium, levcromakalim-evoked relaxations were reduced to a similar level to those obtained in noradrenaline-contracted tissues in the presence of glibenclamide, whereas membrane hyperpolarization was abolished at this time. This finding directly demonstrates that the attenuation of KCO-evoked relaxation observed in the presence of high concentrations of potassium is due to a reduced hyperpolarization (Masuzawa *et al.*, 1990; Parsons *et al.*, 1991a,b), and also supports the contention that a change in smooth muscle membrane potential represents the driving force for relaxation to this agent. This is an important observation, because the reduced relaxation in tissues preconstricted with high concentrations of potassium had been suggested to reflect functional antagonism, i.e. the high level of tone induced by potassium directly reducing the efficacy of the relaxing agent (Cook & Small, 1991). This is clearly not the case in the basilar artery. However, the finding that higher concentrations of levcromakalim can evoke changes in tone which are independent of an increase in membrane potential also indicates another, as yet undefined mechanism may also be involved, possibly an alteration in intracellular smooth muscle calcium handling (Bray *et al.*, 1988; 1991).

In summary, smooth muscle hyperpolarization to both acetylcholine and levcromakalim is abolished by glibenclamide. However, although the relaxation to levcromakalim is also almost totally blocked, relaxation to acetylcholine is not altered. This supports other evidence that endothelium-dependent hyperpolarization to cholinomimetics is not an important mechanism for relaxation in the basilar artery. In addition, NO *per se* provides no stimulus for hyperpolarization, as even saturated solutions of this agent failed to modify the membrane potential in this vessel.

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