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Contribution of both nitric oxide and a change in membrane potential to acetylcholine-induced relaxation in the rat small mesenteric artery

Gareth J. Waldron & [1]Christopher J. Garland

Department of Pharmacology, University of Bristol, University Walk, Bristol BS8 1TD

1 Acetylcholine stimulated repolarization and relaxation in isolated segments of rat small mesenteric artery ($D_{100} = 325 \pm 9\,\mu m$) in which the smooth muscle cells were depolarized and contracted by submaximal concentrations of noradrenaline (0.75–2.5 μM). There was no significant difference either in the time taken to initiate relaxation or hyperpolarization, or for these parameters to reach maximum in response to acetylcholine.

2 The nitric oxide synthase inhibitor, N^G-nitro L-arginine methyl ester (L-NAME, 100 μM) reduced the pD_2 for acetylcholine-induced relaxation from 7.5 to 7 and depressed the maximum relaxation from 89% to 68% in tissues stimulated with noradrenaline. The pD_2 for smooth muscle repolarization in these experiments was also reduced (7.4 to 6.6) but the maximum change in membrane potential in response to acetylcholine was unaltered. The increase in potential now clearly preceded relaxation by 3.7 s (to initiation) and 4.7 s (to maximum).

3 In the presence of noradrenaline and a raised potassium concentration (25 mM), the repolarization to acetylcholine was markedly attenuated. Simultaneous tension measurements also revealed a marked reduction in the maximal relaxation to acetylcholine, but the pD_2 was unchanged at 7.4.

4 The residual relaxation recorded in the absence of marked repolarization (in the presence of noradrenaline and 25 mM potassium) was abolished by the addition of 100 μM L-NAME.

5 Nitric oxide gas in solution (0.2–2.2 μM; NO_g) relaxed artery segments precontracted with noradrenaline. The magnitude of relaxation to NO_g was not altered in the presence of noradrenaline and 25 mM potassium.

6 These data provide additional evidence that acetylcholine-evoked endothelium-dependent increases in membrane potential provide a major mechanism for smooth muscle relaxation in the mesenteric artery. They also show that voltage-dependent and independent (initiated by NO) mechanisms can both contribute to relaxation, and suggest that NO may modulate the increase in membrane potential or the release of a hyperpolarizing factor.

Keywords: Vascular smooth muscle; endothelial cells; nitric oxide; EDHF; mesenteric artery

Introduction

Acetylcholine and related cholinomimetics stimulate endothelium-dependent smooth muscle relaxation which is associated with membrane hyperpolarization (Bolton *et al.*, 1984; Chen *et al.*, 1988; Feletou & Vanhoutte, 1988; Brayden, 1990; McPherson & Angus, 1991; Garland & McPherson, 1992; Rand & Garland, 1992). The smooth muscle hyperpolarization, like the relaxation, is apparently mediated by the release of a diffusible factor (Feletou & Vanhoutte, 1988; Chen *et al.*, 1991). Whether or not nitric oxide (NO), which is, or is very closely related to, endothelium-derived relaxant factor (EDRF), contributes to the membrane potential change in some arteries is not clear. In the rat aorta and pulmonary artery, the hyperpolarization to acetylcholine was not blocked by either oxyhaemoglobin or methylene blue, while relaxation was reduced. These data indicated the involvement of a distinct hyperpolarizing factor (EDHF) released at the same time as NO (Chen *et al.*, 1988; Taylor & Weston, 1988). This suggestion was supported by the failure of exogenous nitric oxide to induce smooth muscle hyperpolarization in concentrations which induced marked relaxation in a number of vascular preparations (Komori *et al.*, 1988; Huang *et al.*, 1988; Brayden, 1990; Rand & Garland, 1992; Plane & Garland, 1993), and the insensitivity of acetylcholine-induced hyperpolarization or repolarization to the action of nitric oxide synthase inhibitors in some arteries (Chen *et al.*, 1991; Nagao & Vanhoutte, 1991; Garland & McPherson, 1992).

In the rat small mesenteric artery, we recently presented evidence against a role for NO in the smooth muscle hyperpolarization and repolarization to acetylcholine (Garland & McPherson, 1992). Although exogenous NO caused glibenclamide-sensitive hyperpolarization in unstimulated smooth muscle cells, this effect was blocked by prior depolarization, while the membrane potential change to acetylcholine was not sensitive to glibenclamide and was increased in magnitude by prior depolarization. In addition, the hyperpolarization (in unstimulated arteries) and the repolarization and relaxation (in noradrenaline-stimulated arteries) to acetylcholine were not markedly altered by either the NO-synthase inhibitor, nitro-L-arginine, or by oxyhaemoglobin. While these results argue strongly against NO having a significant role in the membrane potential increases to acetylcholine, the possibility remains that NO does contribute to the smooth muscle relaxation. If NO and a hyperpolarizing factor are released simultaneously in response to acetylcholine, they may act in parallel to cause relaxation by different mechanisms, one independent of a change in membrane potential, the other dependent upon a change. Even if the production of NO were completely blocked, marked relaxation could still be caused by increases in membrane potential. To study the possibility that NO contributes to relaxation it is therefore necessary to block the increases in membrane potential. Endothelium-dependent hyperpolarization reflects an increase in smooth muscle potassium conductance and can be abolished by raising the external potassium

[1] Author for correspondence.

concentration (Taylor & Weston, 1988; Chen & Suzuki, 1989; Nagao & Vanhoutte, 1991).

In the present experiments, we have investigated the possibility that both NO and increases in smooth muscle membrane potential contribute to the endothelium-dependent relaxation that is induced by acetylcholine in the rat small mesenteric artery. Some of the results have been presented in preliminary form at the Cambridge meeting of the British Pharmacological Society (Waldron *et al.*, 1993).

Methods

Sprague-Dawley rats of either sex (approximately 300 g) were stunned and killed by cervical dislocation. The mesentery was removed and placed in physiological salt solution (PSS) at room temperature. A segment of a third order branch of the superior mesenteric artery was carefully removed and cut into cylindrical segments 1–2 mm in length. Segments were then mounted in a tissue chamber for recording simultaneous changes in smooth muscle membrane potential and tension, as previously described (Garland, 1987). Briefly, two tungsten wires (each of 25 μm diameter) were passed through the segment's lumen and each wire attached to a small plastic foot. One foot was coupled to an isometric force transducer (Harvard Biosciences, 52-9529) and the other to a microdrive (Prior, code 71). After 60 min equilibration, a passive diameter-tension curve was constructed, from which the effective transmural pressure was calculated. The segment was then set at a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg (D_{100}; Mulvany & Halpern, 1977). The segment was superfused (at 3–5 ml min^{-1}) with PSS which had been gassed with a mixture of 95% O_2/5% CO_2 and warmed to 37°C. Concentration-response curves were constructed from responses to cumulative concentrations of acetylcholine in segments precontracted to noradrenaline (0.25–2.5 μM). Nitric oxide synthase blocking drugs increase the size of contraction to a given concentration of spasmogen (Cocks & Angus, 1991). Thus, in experiments in which N^G-nitro L-arginine methyl ester (L-NAME) was present, the concentration of noradrenaline was decreased in an attempt to maintain impalements and obtain a similar level of tone compared to the experiments in which L-NAME was absent. Where this was not successful, control experiments were performed in which similar levels of tone were induced. All drugs were equilibrated with the superfusate before it entered the tissue chamber apart from nitric oxide gas – PSS containing the gas (usually 10–100 μl) was injected close to the artery segment from a gas-tight syringe.

Electrophysiology

Measurement of smooth muscle membrane potential was made with a glass microelectrode, advanced through the adventitial surface of the artery segment. The electrodes were filled with 2 M KCl and had resistances of 80–120 MΩ. Membrane electrical events were recorded through a high-input impedance d.c. preamplifier (Neurolog NL102G) and digitized, together with data from the isometric transducer, and stored on disc.

Solutions and drugs

Experiments were performed in physiological salt solution (PSS) of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, disodium EDTA 0.027 and glucose 11.

Drugs used were acetylcholine chloride (BDH); (−)-noradrenaline bitartrate (arterenol, Sigma); N^G-nitro L-arginine methyl ester (Sigma); nitric oxide gas (research grade, BDH).

Preparation of nitric oxide solution

One ml of 99% nitric oxide gas was injected, using a gas tight syringe, into a gas tight ampoule containing 100 ml of PSS which had been bubbled with research grade helium (BOC) for 45–60 min. Nitric oxide solution (NO_g) was then injected into the tissue chamber in volumes of 10–100 μl, with a gas tight syringe. Control injections of helium gassed PSS were always performed to assess the extent of potential injection artifacts.

Analysis of data

Where oscillations in tension or membrane potential occurred, the response to the agonist was taken to be the level of tension or membrane potential at half the amplitude of the oscillations. Relaxations are expressed as a percentage decrease in the initial tone to noradrenaline. Other 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.

Results

Acetylcholine-induced changes in tension and membrane potential of mesenteric artery segments

The resting membrane potential of smooth muscle cells in the rat small mesenteric artery (D_{100} 325 ± 8.6 μm; $n = 23$) was −61.6 ± 2.1 mV ($n = 11$ cells). Noradrenaline (0.75–2.5 μM) depolarized the cells to −44.5 ± 2.0 mV ($n = 7$), with a subsequent increase in active tension to 1.41 ± 0.17 mN mm^{-1} ($n = 12$). In 78% of experiments, rhythmic oscillations in membrane potential and tension developed in the continued presence of noradrenaline (14.2 ± 0.5 cycles min^{-1}, $n = 11$). The application of acetylcholine (0.01–1 μM) stimulated concentration-dependent repolarization and relaxation (Figure 1). The pD_2 for relaxation was 7.5 ± 0.05 and the

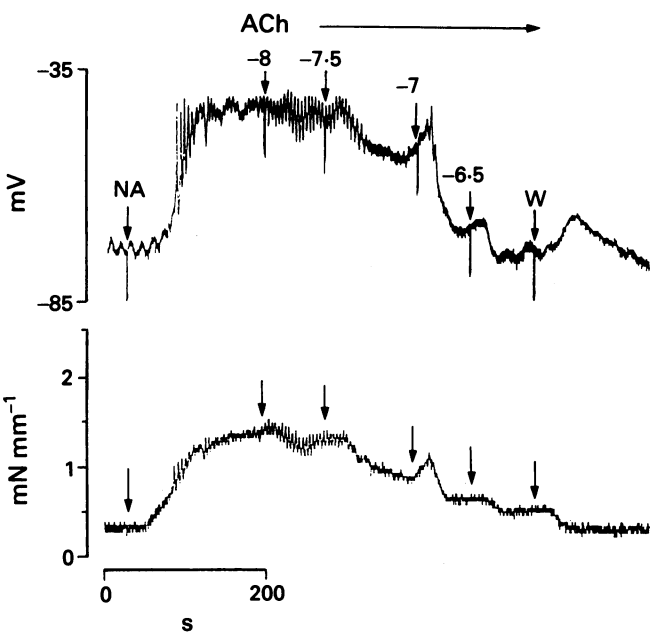

Figure 1 Representative experimental trace showing simultaneous recording of smooth muscle membrane potential (upper trace) and tension (lower trace). Noradrenaline (NA; 1 μM) depolarizes and contracts the cells and the subsequent addition of acetylcholine (ACh; 0.01–0.3 μM) stimulates concentration dependent repolarization and relaxation. Drugs were added to the superfusate chamber at the arrows. Washout is indicated by W.

maximum relaxation obtained was 92.1 ± 2.0% (all $n = 7$) with 1 μM (Figure 2a). Above 1 μM, acetylcholine stimulated an endothelium-independent smooth muscle contraction. The pD_2 for membrane repolarization was not significantly different at 7.4 ± 0.04 and the maximum increase in membrane potential was around 21 mV, changing the potential to − 66.9 ± 3.4 mV (all $n = 4$; Figure 2b). There was no difference in either the time taken to initiate relaxation or repolarization after the application of acetylcholine (relaxation preceded repolarization by 1.1 ± 1.8 s; $n = 21$), or the time taken to reach maximum (relaxation preceded repolarization by 1.6 ± 3.2 s; $n = 20$).

Acetylcholine-induced repolarization and relaxation in the presence of L-NAME

L-NAME (100 μM) was applied to the superfusate 30 min before noradrenaline and was present throughout the subsequent application of acetylcholine. In the presence of L-NAME, the concentration-response curve for relaxation was shifted to the right and the maximal relaxation reduced. The pD_2 changed from 7.5 ± 0.05 to 6.96 ± 0.12 ($P < 0.001$) and the maximal relaxation was reduced from 88.6 ± 5.1% to 67.6 ± 7.5% at 1 μM (all $n = 4$; Figure 2a). As in the absence of L-NAME, endothelium-independent contractions were obtained to higher concentrations of acetylcholine. With repolarization the pD_2 was also changed, from 7.4 ± 0.04 to 6.6 ± 0.13 ($P < 0.001$, $n = 4$; Figure 2b). L-NAME did not alter the amplitude of the maximal repolarization to acetylcholine, but it did alter the temporal relationship between increases in membrane potential and relaxation. Although in the absence of L-NAME there was no difference in the mean time taken to initiate repolarization and relaxation, or reach a maximal effect, in the presence of L-NAME the onset of repolarization always preceded the onset of relaxation by 3.7 ± 1.2 s and maximal repolarization was reached before maximal relaxation by 4.2 ± 1.8 s (both $n = 11$). In contrast to the experiments described above, in the presence of L-NAME rhythmic oscillations in membrane potential and tension were not observed indicating that NO may be involved in these oscillations, as suggested by Gustaffson *et al.* (1993).

Acetylcholine responses in the presence of raised extracellular potassium

Raising the concentration of potassium in the superfusate from 4.7 to 25 mM in the presence of prior smooth muscle depolarization and contraction to a submaximal concentration of noradrenaline, caused an additional increase in tension (0.52 mN mm^{-1}) and a depolarization of 13 mV (Table 1). Under these conditions, the subsequent addition of

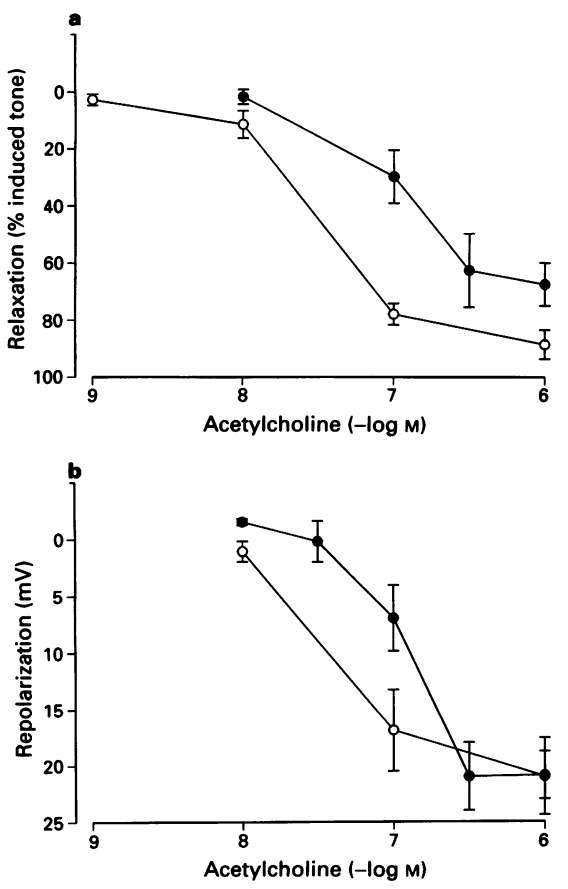

Figure 2 Mean concentration-effect curves constructed to acetylcholine in the absence (○) and presence (●) of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM). (a) Relaxation of noradrenaline-induced contraction. (b) Reversal of noradrenaline-induced depolarization recorded simultaneously with the relaxations summarized in (a). Results are the mean ± s.e.means where they exceed symbol size ($n = 4$).

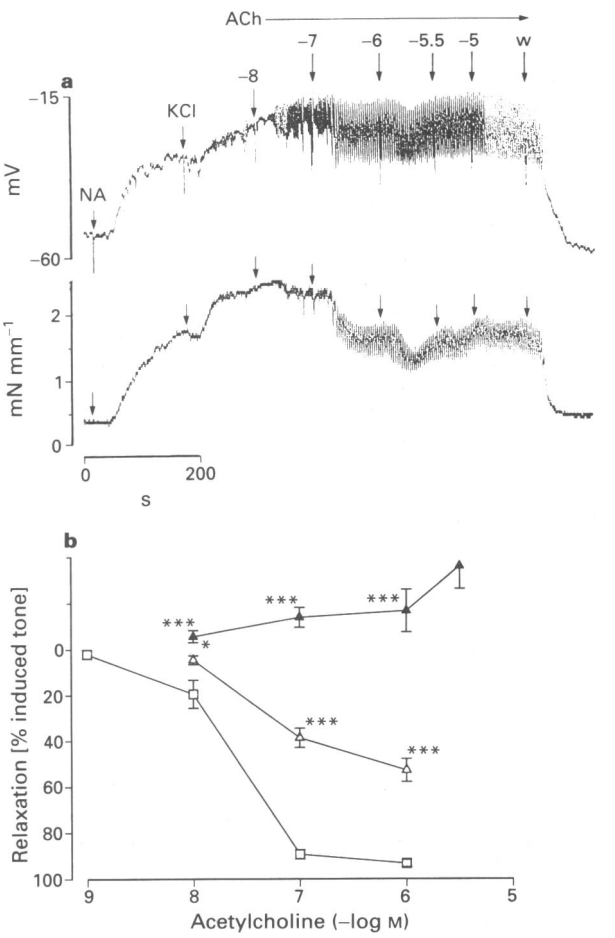

Figure 3 (a) Representative trace of a simultaneous record of membrane potential (upper trace) and tension (lower trace) showing the lack of any measurable repolarization and a marked reduction in the amount of relaxation to acetylcholine (ACh) in cells depolarized and contracted in the combined presence of 25 mM potassium and noradrenaline (NA). Drugs were added to the superfusate chamber at the arrows. Washout of all drugs is indicated by W. (b) Mean concentration-effect curves showing the effect of 25 mM potassium (△) and the combination of 25 mM potassium and 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) (▲) on the relaxation to acetylcholine (□) in the presence of noradrenaline. Values are means ($n = 4$–11) with s.e.means. $P < 0.05$*; $P < 0.001$*** compared to control tissues.

Table 1 The effect of N^G-nitro-L-arginine methyl ester (L-NAME) and raised extracellular potassium on noradrenaline induced contraction and depolarization in rat small mesenteric artery

	Resting membrane potential (mV)	*Plus noradrenaline* *Membrane potential* (mV)	*Tension* ($mN\ mm^{-1}$)	*Concentration range noradrenaline* (μM)
Control	-61.6 ± 2.1 $n=11$	-44.5 ± 2.0 $n=7$	1.41 ± 0.17 $n=12$	0.75–2.5
L-NAME	-58.5 ± 2.2 $n=4$	-40.8 ± 2.2 $n=5$	1.81 ± 0.20 $n=8$	0.75–1
K^+ 25 mM	-57.2 ± 1.6 $n=4$	-31.6 ± 3.8 $n=4$**	1.93 ± 0.14 $n=5$	1–2.5
K^+ 25 mM and L-NAME	-59.2 ± 7.1 $n=3$	-33.8 ± 9.0 $n=3$	2.05 ± 0.14 $n=12$**	0.25–1

As nitric oxide synthase blocking drugs increase the magnitude of contraction the concentration of noradrenaline was varied (right-hand column) to as far as possible induce comparable levels of tone. Control experiments showed no functional antagonism between higher levels of tone (around 2 $mN\ mm^{-1}$) and relaxation to acetylcholine. Values are mean ± s.e.mean. Significant difference from control values $P<0.01$**.

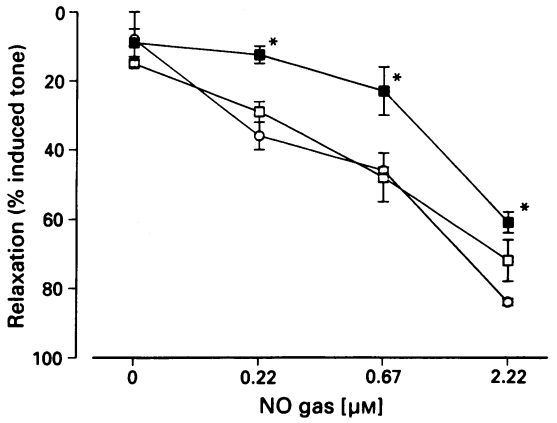

Figure 4 Mean concentration-effect curves showing the effect of raising external potassium from control levels of 4.7 mM (○) to 25 mM (□) or 30 mM (■), on the ability of nitric oxide gas (NO_g) injected directly into the organ bath to relax noradrenaline precontracted tissues. Values are means ($n=3$) ± s.e.mean. $P<0.05$* compared to 4.7 mM K^+ Krebs.

acetylcholine stimulated only a small increase in membrane potential (cf. 4 mV; Figure 3a). Rhythmic oscillations of the membrane potential and tension were still observed under these conditions (frequency 16.8 ± 0.6 cycles min^{-1}; $n=4$). The maximum smooth muscle relaxation to acetylcholine was markedly attenuated to $53.8 \pm 5.0\%$ and the pD_2 was not different from control values at 7.4 ± 0.07 ($n=5$). The residual relaxation to acetylcholine was abolished by incubating the artery segments with 100 μM L-NAME for 30 min prior to the addition of noradrenaline and increased potassium (Figure 3b). The contraction to noradrenaline and potassium in this series was close to 2 $mN\ mm^{-1}$ and the membrane potential was depolarized to −34 mV (Table 1). In experiments without L-NAME present, the potency and maximal relaxation to acetylcholine was not altered when applied against a similar level of noradrenaline-induced tone (2.04 ± 0.1 $mN\ mm^{-1}$; $n=3$). After washout and reincubation in a Krebs solution containing a normal potassium concentration, acetylcholine stimulated a transient response in the continuing presence of noradrenaline and L-NAME (six of eight experiments).

Effect of exogenous nitric oxide

Relaxation to nitric oxide (NO_g) injected directly into the tissue chamber, in artery segments contracted to noradrenaline, was not modified by the presence of 25 mM potassium. However, the magnitude of relaxation to NO_g was significantly reduced in the presence of noradrenaline and 30 mM potassium (Figure 4).

Discussion

The main conclusions from the work presented in this paper are that an increase in the smooth muscle membrane potential in response to acetylcholine provides a major drive to relaxation in the mesenteric artery, and also that nitric oxide is released in response to acetylcholine and contributes to relaxation by a mechanism independent of membrane potential change.

A close correlation between acetylcholine-induced increases in smooth muscle membrane potential and relaxation has been reported previously. Also, in unstimulated tissues, acetylcholine was shown to induce smooth muscle hyperpolarization. Both these responses to acetylcholine were endothelium-dependent (McPherson & Angus, 1991; Garland & McPherson, 1992). We have extended these observations in the present study, using simultaneous measurements of membrane potential and tension, to show that the concentration of acetylcholine required to change membrane potential and tension are not different. In addition, when the production of NO was blocked by the nitric oxide synthase inhibitor L-NAME, the onset of the change in membrane potential and the time to peak response always clearly preceded the accompanying changes in smooth muscle tension. In the absence of L-NAME there was no clear temporal relationship between these parameters.

Acetylcholine stimulates endothelium-dependent increases in smooth muscle potassium conductance, an effect which presumably underlies the increase in membrane potential observed in recent studies (Chen *et al.*, 1988; Chen & Suzuki, 1989). This idea is supported by the effect of raising the external concentration of potassium from 4.7 mM to 25 mM, which almost abolished the repolarization to acetylcholine and at the same time reduced the total relaxation by nearly 50%. This concentration of potassium did not reduce the relaxation to NO_g, so it would not be expected to modify the relaxant action of any NO released from the endothelium. Interestingly, higher concentrations of potassium did appear to reduce the relaxation to exogenous NO. An explanation for this effect is not apparent, as the levels of contraction were similar with both concentrations of potassium. This observation was not investigated further. Potassium (25 mM) would not be expected to reduce the production of NO by the endothelium as concentrations of 65 mM did not reduce endothelium-dependent relaxation to acetylcholine in the rabbit basilar artery which is predominantly due to the action of NO (Plane & Garland, 1993). So the fact that membrane

potential change in response to acetylcholine was closely correlated to smooth muscle relaxation, and that in the absence of a change in membrane potential of more than a couple of millivolts relaxation was reduced by around 50%, highlights the importance of voltage-sensitive mechanisms in the relaxant responses of the small mesenteric artery to this cholinomimetic.

Although an increase in the smooth muscle membrane potential appears to have a major role in causing relaxation to acetylcholine, several observations indicate that the increase does not reflect an action of NO *per se*. First, although exogenous NO_g or acidified sodium nitrite did stimulate smooth muscle hyperpolarization in the mesenteric artery, this action was blocked by prior depolarization to noradrenaline. In contrast, depolarization to noradrenaline increased rather than decreased the magnitude of the repolarization to acetylcholine and, in depolarized cells, NO stimulated marked smooth muscle relaxation in the absence of a change in membrane potential. Second, the hyperpolarization to NO was reversibly inhibited in the presence of the potassium channel blocker, glibenclamide. However, glibenclamide did not affect either the acetylcholine-induced hyperpolarization or the repolarization and relaxation in this artery. Third, neither repolarization nor relaxation to acetylcholine was blocked with either nitro-arginine or oxyhaemoglobin, although oxyhaemoglobin totally abolished the responses to exogenous NO (Garland & McPherson, 1992).

In light of the discrepancies between the membrane responses to exogenous NO and acetylcholine, the observations clearly indicate that changes in membrane potential to the latter do not reflect a direct action of NO on the smooth muscle cells in the mesenteric artery. Presumably, they are explained by the release of a separate diffusible factor, as demonstrated in the canine femoral and guinea-pig carotid arteries (Feletou & Vanhoutte, 1988; Chen *et al.*, 1991). Evidence for the action of a separate factor, EDHF, has been obtained in a large number of different vascular preparations (Chen *et al.*, 1988; Feletou & Vanhoutte, 1988; Taylor & Weston, 1988; Chen & Suzuki, 1989; Chen *et al.*, 1991; Garland & McPherson, 1992; Rand & Garland, 1992; Plane & Garland, 1992). Studies on the relative contribution which membrane potential changes make to relaxation are, however, limited. In the canine coronary artery, membrane potential changes to acetylcholine could be blocked with 60 mM potassium, an effect associated with a reduction in relaxation by around 60% (Nagao & Vanhoutte, 1991). As in the present study, the residual relaxation was abolished after inhibition of NO synthase. Based on the persistence of variable sized relaxations to acetylcholine in the presence of nitro-arginine in different arteries from the rat, it has been suggested that EDHF may make a greater contribution than EDRF to the relaxant response in small compared to large arteries (Nagao *et al.*, 1992). Studies with rabbit large cerebral arteries lend support to this suggestion, as the acetylcholine-induced relaxation in the basilar artery appears predominantly to reflect an action of NO (Rand & Garland, 1992; Plane & Garland, 1993).

While around half of the total relaxation to acetylcholine can be explained by a change in the smooth muscle membrane potential, the remainder appears to reflect a contribution from NO. Our original hypothesis that blocking hyperpolarization may reveal a contribution from NO was correct, as the residual relaxation in the absence of hyperpolarization was totally abolished by L-NAME. Our results are also in agreement with the observations of Gustafsson *et al.* (1993), who found that nitro-arginine and other manipulations to inhibit the action of NO blocked the rhythmic tension oscillations induced by noradrenaline in the mesenteric artery. However, we were also able to demonstrate an action of L-NAME against both relaxation and repolarization in smooth muscle cells in a normal potassium concentration. If the interpretation above is correct, and NO is not responsible for the change in membrane potential to acetylcholine, the shift in the curve for increases in membrane potential with L-NAME suggests that NO can modulate the response. A similar effect was observed with NO synthase inhibitors in the rabbit basilar artery (Rand & Garland, 1992). NO may modulate the release of an EDHF from the endothelium or act, either directly or indirectly via protein kinases, to modify changes in smooth muscle potassium conductance. Whatever the precise explanation, an effect of L-NAME was in contrast to our previous study, where nitro-arginine did not alter either smooth muscle repolarization or relaxation in similar size mesenteric arteries from Wistar-Kyoto rats (Garland & McPherson, 1992).

An explanation for the apparent discrepancy between the two studies is not obvious. The discrepancy is unlikely to reflect the use of L-NAME in the present study, rather than nitro-arginine, as similar sized relaxations to acetylcholine also persisted in the presence of nitro-arginine in larger diameter mesenteric arteries (Nagao *et al.*, 1992). In similar sized mesenteric arteries, nitro-arginine did not apparently reduce relaxation, but modified the response which became much more transient (Gustaffson *et al.*, 1993) suggestive of a response to EDHF (Chen & Suzuki, 1989). One possible explanation is that the variable effect of NO synthase inhibitors reflects the strain of rats used. Gustaffson *et al.* (1993) failed to find any effect with nitro-arginine in a number of their experiments with mesenteric arteries from Wistar rats, whilst it has been reported that nitro-arginine blocks acetylcholine-induced relaxation incompletely in mesenteric arteries from Sprague-Dawley rats (this study; Wu *et al.*, 1993). In the isolated perfused mesenteric bed from the colony of Sprague-Dawley rats used in the present study, we have found that the vasodilatation to acetylcholine is reduced by a similar extent, around 20–30%, with either nitro-arginine or its methyl ester (Parsons *et al.*, 1993). However, this reduction was observed in 80% of cases. In the remaining experiments, NO synthase inhibitors had no effect on the vasodilatation (Parsons *et al.*, unpublished data). The persistence of relaxation and changes in membrane potential to acetylcholine, in arteries from the inbred Wistar-Kyoto strain (Garland & McPherson, 1992) may be the functional expression of one aspect of this variability.

In summary, these data show that acetylcholine stimulates both voltage-dependent and independent mechanisms in the rat small mesenteric artery. The repolarization to acetylcholine, which is known to be endothelium-dependent, can account for approximately 50% of the relaxation to this agent. The remainder of the endothelium-dependent relaxation appears to be stimulated by NO, based on the sensitivity of the residual relaxation (in 25 mM potassium) to the NO synthase inhibitor L-NAME.

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