British Journal of Pharmacology (2001) 133, 665–672

www.nature.com/bjp

Relaxation to authentic nitric oxide and SIN-1 in rat isolated mesenteric arteries: variable role for smooth muscle hyperpolarization

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1 Authentic nitric oxide (NO; 0.1–10 μmoles) caused transient, dose-dependent relaxation of phenylephrine-induced tone without changing membrane potential in mesenteric arteries. Larger doses, above 10 μmoles, did not evoke more relaxation (maximal relaxation to 150 μmoles NO in denuded arteries, 69±7%, $n=8$) but stimulated muscle hyperpolarization (maximum 19±3 mV, $n=5$).

2 The soluble guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10 μM), abolished relaxation to low doses of NO ($n=4$), but did not modify hyperpolarization with higher doses of NO ($n=4$). The potassium channel blocker charybdotoxin (ChTX; 50 nM) abolished hyperpolarization to high doses of NO and significantly reduced the maximal relaxation (to 43±6%, $n=4$; $P<0.01$). ODQ and ChTX together abolished tension and membrane potential change to all doses of NO ($n=4$).

3 All relaxations to 3-morpholino-sydnonimine (SIN-1; 0.01–10 μM) were associated with hyperpolarization. When the endothelium was intact, ChTX inhibited hyperpolarization and relaxation to SIN-1 ($n=5$), while iberiotoxin (IbTX; 50 nM) or 4-aminopyridine (4-AP; 500 μM) reduced relaxation by 40% and 20%, respectively and by 80% in combination ($n=6$ in each case).

4 In denuded arteries, relaxation to SIN-1 was unaffected by either ChTX or ODQ alone, but abolished by the inhibitors together ($n=6$). Alone, 4-AP did not alter relaxation, but in the presence of ODQ it reduced the maximal response by around 45% ($n=6$; $P<0.01$). 4-AP, ODQ and IbTX together inhibited relaxation to SIN-1 by 75% ($n=6$; $P<0.01$).

5 Therefore, cyclic guanosine 3′,5′-monophosphate (cyclic GMP)-independent smooth muscle hyperpolarization, possibly involving direct activation of calcium-activated and voltage-sensitive potassium channels, contributes to relaxation evoked by authentic NO and SIN-1. However, the importance of each pathway depends on the source of NO and with SIN-1 the relative contribution from each pathway is modified by the endothelium.

British Journal of Pharmacology (2001) **133,** 665–672

Keywords: Nitric oxide; potassium channels; soluble guanylyl cyclase; vascular smooth muscle

Abbreviations: 4-AP, 4-aminopyridine; BK_{Ca}, large-conductance calcium-activated potassium channels; cyclic GMP, cyclic guanosine 3′,5′-monophosphate; ChTX, charybdotoxin; K_{DR}, delayed rectifier potassium channels; IbTX, iberiotoxin; NO, nitric oxide; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; SIN-1, 3-morpholino-sydnonimine

Introduction

Smooth muscle relaxation to nitric oxide (NO) is thought to be explained by the activation of soluble guanylyl cyclase leading to an increase in cytoplasmic concentrations of cyclic guanosine-3′,5′-monophosphate (cyclic GMP; Ignarro, 1991). cyclic GMP then activates specific cyclic GMP-dependent protein kinases, which act in a number of ways to decrease the cytoplasmic calcium concentration (Cornwell *et al*., 1991) and reduce the sensitivity of the contractile myofilaments (Tran *et al*., 1998).

One effect of cyclic GMP is to activate charybdotoxin (ChTX)-sensitive potassium channels, leading to hyperpolarization of the smooth muscle membrane potential and reducing calcium influx through voltage-sensitive channels (Robertson *et al*., 1993). In addition, authentic NO has been shown to activate directly ChTX-sensitive potassium channels in isolated smooth muscle cells from a number of vessels, including rabbit aorta and rat mesenteric arteries (Bolotina *et al*., 1994; Mistry & Garland, 1998). Therefore, it is not surprising that NO can evoke smooth muscle hyperpolarization in arterial smooth muscle (Tare *et al*., 1990; Garland & McPherson, 1992; Murphy & Brayden, 1995), and that in a number of preparations, relaxation to NO can be inhibited with ChTX (Khan *et al*., 1993; Bolotina *et al*., 1994; Archer *et al*., 1995, Plane *et al*., 1996; Cohen *et al*., 1997).

The extent to which smooth muscle relaxation follows the activation of potassium channels by NO, and the involvement of cyclic GMP, is not clear. It may in fact vary both with the arterial preparation and the source of the NO. In the rabbit

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isolated carotid artery, authentic NO and the NO donors, 3-morpholino-sydnonimine (SIN-1) and S-nitroso-N-acetylpenicillamine, evoked smooth muscle relaxation and hyperpolarization with a similar potency. In each case, inhibition of membrane hyperpolarization significantly reduced relaxation. However, the contribution of cyclic GMP to the changes in membrane potential and tension differed, such that inhibiting the generation of cyclic GMP effectively abolished both the hyperpolarization and relaxation to the NO donors, while the equivalent responses to authentic NO were only reduced by about 40%. The persistent relaxation and hyperpolarization to NO was abolished with ChTX, suggesting it may directly activate the potassium channels (Cohen *et al*., 1997).

A very different mechanistic profile appears to exist in the rat isolated mesenteric artery. In this small resistance artery, bolus doses of authentic NO (0.1 – 1 μmol) reversibly hyperpolarized the smooth muscle resting potential *via* a glibenclamide-sensitive mechanism, but relaxed cells pre-stimulated with noradrenaline without a change in membrane potential (Garland & McPherson, 1992). In endothelium-intact segments of the mesenteric artery, the NO-donor, SIN-1, evoked smooth muscle relaxation which was abolished with ChTX, but was unaffected by the guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; Garthwaite *et al*., 1995; Plane *et al*., 1996). However, in mesenteric arteries denuded of endothelium, relaxation to SIN-1 was only partially inhibited with either ChTX or ODQ, but blocked completely when these two agents were applied simultaneously (Plane *et al*., 1996).

The aim of the present study was to extend these observations to investigate further the relative importance of a change in membrane potential to the relaxation in rat isolated mesenteric arteries stimulated with either authentic NO or the NO donor, SIN-1.

Methods

Tension measurements

Male Wistar rats (250 – 300 g) were stunned and then killed by cervical dislocation. Segments of third order branches of the superior mesenteric artery ($D_{100} = 290 \pm 15$ μm; $n = 40$) were mounted in a Mulvany-Halpern myograph under a normalized tension as described previously (Plane *et al*., 1996). Briefly, the segments of artery were mounted between two tungsten wires (25 μm in diameter) and maintained in a static bath at 37°C in oxygenated Krebs buffer, containing indomethacin (2.8 μM).

Cumulative concentration-response curves were constructed to SIN-1 in arterial segments depolarized and constricted with phenylephrine (1 – 3 μM). Authentic NO was applied as bolus doses. In some experiments, the endothelial cell layer was removed by gently rubbing the intimal surface with a hair and successful removal of the endothelium confirmed by the absence of relaxation to acetylcholine (1 μM). In all experiments, the concentration of phenylephrine was adjusted to give a similar level of tone in the presence and absence of inhibitors (mean depolarization and contraction: 28 ± 5 mV ($n = 10$ cells from six preparations) and 15 ± 3 mN ($n = 14$), respectively).

Electrophysiology

Measurement of smooth muscle membrane potential was made with a glass microelectrode advanced through the adventitial surface of the arterial segment. The electrodes were back filled with 2 M KCl and had resistances of 60 – 120 MΩ. Membrane electrical events were recorded through a high impedance d.c. pre-amplifier (Neurolog 102G) and together with data from the isometric force transducer, stored on disc (MacLab, AD Instruments, Hastings, U.K.).

Solutions and drugs

Tissues were maintained in Krebs buffer of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.18, glucose 11, disodium EDTA 0.027 and $CaCl_2$ 2.5. All drugs were from Sigma except for SIN-1 (Tocris), ODQ (Tocris), Iberiotoxin (IbTX; Calbiochem) and ChTX (Calbiochem). All drugs were dissolved in Krebs buffer except for ODQ which was dissolved in DMSO, and indomethacin which was dissolved in 2% Na_2CO_3.

Preparation of NO solutions

Solutions of NO were prepared by injecting research grade NO gas (BDH) into de-gassed Krebs buffer as described previously (Plane *et al*., 1998). NO solutions were injected in to the myograph (bath volume 10 mls) close to the segments of artery and in volumes of less than 250 μl. Control injections of Krebs solution were made to assess the extent of any injection artefacts.

Analysis of data

Arterial relaxation is expressed as a percentage decrease in the phenylephrine-induced contraction and smooth muscle hyperpolarization either in mV or as a percentage reversal of phenylephrine-induced depolarisation. All data are expressed as mean $\pm$ s.e.mean and the significance of differences between mean values calculated using the paired Students *t*-test.

Results

Membrane potential and tension responses to authentic NO solutions

Bolus additions of authentic NO (0.1 – 10 μmoles) caused transient, dose-dependent relaxation in arterial segments pre-stimulated with phenylephrine (1 – 3 μM), without altering significantly the smooth muscle membrane potential. In endothelium-denuded arteries, a maximum reversal of contraction (70 ± 7%; $n = 8$) was achieved with 10 μmoles of authentic NO. With higher doses of NO, up to 150 μmoles, the amplitude of NO-evoked relaxation did not increase but smooth muscle hyperpolarization was now evident. The maximal relaxation and hyperpolarization obtained with 150 μmoles of NO was 69 ± 7% ($n = 8$) and 19 ± 3 mV ($n = 5$), respectively. Representative traces showing simultaneous changes in membrane potential and tension to bolus doses of NO in an endothelium-denuded arterial segment are shown in Figure 1a.

Application of ODQ (10 μM; 10 min), an inhibitor of soluble guanylyl cyclase, abolished the relaxation to lower doses of NO (0.1–5 μmoles; $n=4$), and significantly reduced relaxation to higher doses (maximal relaxation to 150 μmoles 50$\pm$5%; $n=5$; $P<0.01$). However, the hyperpolarizations which accompanied relaxations to 10–150 μmoles of NO were not altered by this inhibitor (14$\pm$2 mV; $n=3$; $P>0.05$). Using radioimmunoassay, this concentration of ODQ has been shown to abolish completely SIN-1-evoked formation of cyclic GMP in this artery (Plane *et al*., 1996). Representative traces showing simultaneous changes in membrane potential and tension to NO in an endothelium-denuded arterial segment in the presence of ODQ are shown in Figure 1b and mean dose-response curves showing the effect of ODQ on relaxation and hyperpolarization to authentic NO are shown in Figure 2a and b, respectively. In the presence of ODQ, the contraction to phenylephrine increased somewhat, although the variability in this effect meant the change was not significant ($n=8$, data not shown). However, the concentration of phenylephrine was varied to ensure the contraction matched that obtained in the absence of ODQ

The potassium channel inhibitor ChTX (50 nM; 10 min) abolished hyperpolarization to higher doses of NO and significantly blunted the accompanying relaxations such that the maximum relaxation to 150 μmoles of NO was only 43$\pm$6% ($n=4$; $P<0.01$). In contrast, relaxations to lower doses were largely unaffected ($n=4$). In the presence of ODQ and ChTX together, tension and membrane potential responses to all doses of NO were abolished ($n=4$). Mean dose-response curves showing the effect of ChTX, alone and with ODQ, on relaxation and hyperpolarization to authentic NO are shown in Figure 2a and b, respectively.

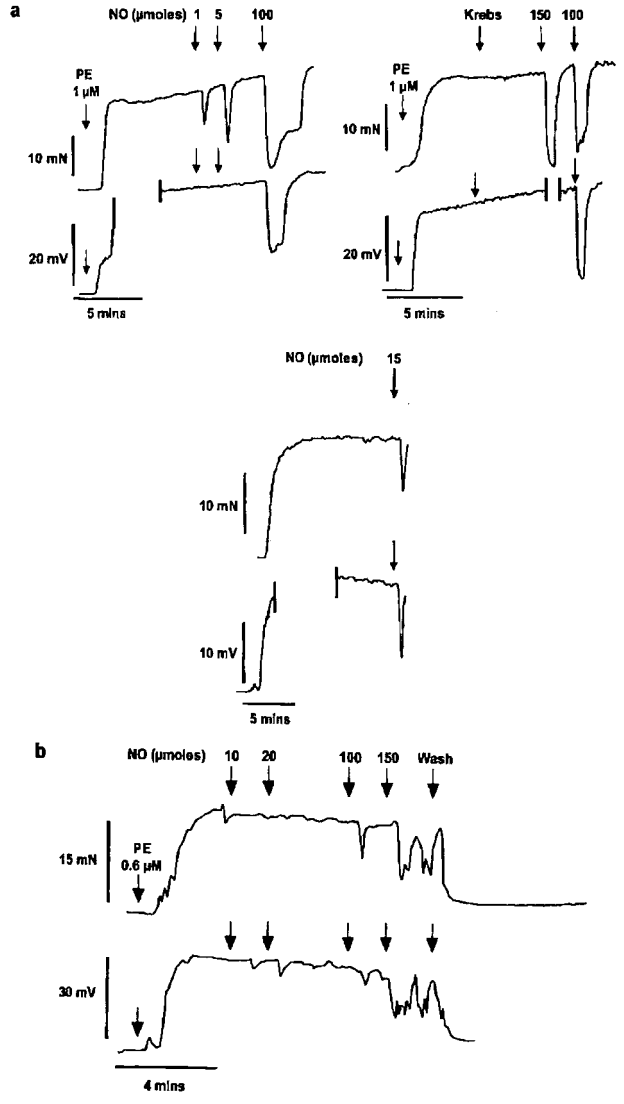

Figure 1 Representative traces showing simultaneous recording of changes in smooth muscle tension (mN) and membrane potential (mV) to bolus doses of authentic NO (1–150 μmoles) in an endothelium-denuded artery segments pre-stimulated with phenylephrine (1 μM) in the absence (a) and presence (b) of ODQ (10 μM). In the presence of ODQ, the concentration of phenylephrine was reduced to 0.6 μM to ensure that a comparable level of pre-contraction and depolarization was achieved. Vertical lines denote loss of electrode impalement.

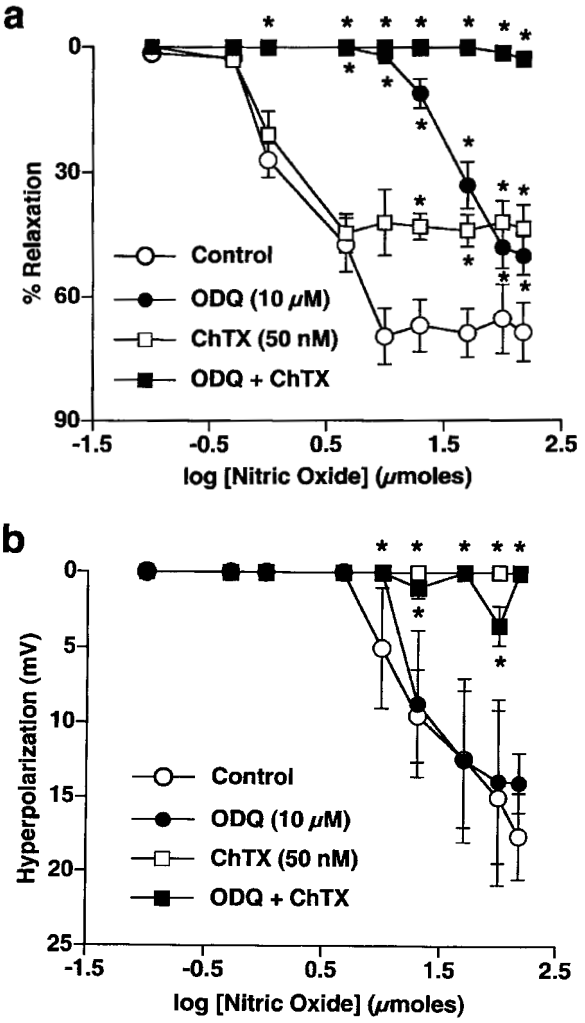

Figure 2 Mean dose-response curves for NO (0.1–150 μmoles)-evoked relaxation (a) and hyperpolarization (b) in the absence and presence of ODQ (10 μM) and ChTX (50 nM) alone and in combination. All points are the mean of 3–8 observations with s.e.means shown by vertical lines. *$P<0.01$ compared to control values.

Bolus additions of NO also evoked transient, dose-dependent relaxations of phenylephrine-induced tone in endothelium-intact arterial segments, although the threshold dose was higher (1 μmole compared to 0.1 μmole) than in denuded arteries ($n=7$). As in denuded vessels, relaxation of intact tissues to higher doses of NO (above 10 μmoles) was accompanied by membrane hyperpolarization, the maximal changes in tension and membrane potential being similar to those observed in the absence of a functional endothelial cell layer ($75\pm8\%$, $n=5$ and 18 ± 3 mV, $n=3$, respectively).

The effect of ODQ (10 μM) and ChTX (50 nM) on responses to authentic NO in endothelium-intact tissues was identical to denuded arteries. ODQ abolished relaxation to lower doses of NO and significantly reduced the maximal relaxation to $45\pm6\%$ ($n=5$). Pre-incubation with ChTX abolished both the hyperpolarization to doses of NO above 10 μmoles ($n=4$) and reduced the maximal relaxation to 150 μmoles to $23\pm7\%$ ($n=4$). Application of ChTX and ODQ together abolished responses to all doses of NO ($n=5$).

Membrane potential and tension responses to SIN-1

The NO donor, SIN-1 (0.01 – 10 μM), elicited concentration-dependent, slow hyperpolarization and relaxation in both endothelium-intact and -denuded arteries pre-stimulated with phenylephrine (1 – 3 μM). There was no significant difference in the threshold concentration (0.03 μM) or EC_{50} values for SIN-1-evoked changes in membrane potential and tension between intact (EC_{50} values; 0.11 ± 0.05 μM, $n=5$ and 0.13 ± 0.05 μM, $n=3$, respectively), and denuded arteries (EC_{50} values; 0.10 ± 0.04 μM, $n=7$ ($P>0.05$) and 0.11 ± 0.05 μM, $n=3$, ($P>0.05$), respectively). Representative traces illustrating simultaneous changes in membrane potential and tension to cumulative additions of SIN-1 (0.01 – 10 μM) in an endothelium-intact arterial segment are shown in Figure 3a.

In intact arterial segments, hyperpolarization and relaxation to SIN-1 was abolished by prior exposure to ChTX (50 nM; 10 min; $n=4$). In contrast, in endothelium-denuded tissues, although hyperpolarization evoked by SIN-1 was abolished by pre-incubation with ChTX ($n=3$), the relaxation was unaffected either by ChTX ($n=6$) or by ODQ (10 μM; 10 min; $n=4$) alone. However, following pre-incubation with ChTX and ODQ together, both the sustained hyperpolarization ($n=4$) and relaxation ($n=7$) of endothelium-denuded arterial segments in response to SIN-1 were abolished although small oscillations in both membrane potential and tension were still observed. Representative traces illustrating simultaneous recordings of changes in membrane potential and tension to cumulative additions of SIN-1 (0.01 – 10 μM) in an endothelium-denuded arterial segment in the presence of ChTX, alone and in combination with ODQ, are shown in Figure 3b and c. Mean concentration-response curves showing the effect of ChTX and ODQ, alone and in combination, on relaxation and hyperpolarization to SIN-1 in denuded vessels are shown in Figure 4a and b, respectively.

Application of either IbTX (50 nM) or 4-aminopyridine (4-AP; 500 μM) alone reduced the maximum SIN-1-evoked relaxation in endothelium-intact strips by around 40% and 20% ($n=6$; $P<0.01$ in each case), respectively. Combination of the two inhibitors reduced the maximal response by around 80% to $15\pm5\%$ ($n=6$; $P<0.01$). Mean concentration-response curves showing the effect of IbTX and 4-AP, alone and in combination, on relaxation to SIN-1 in endothelium-intact vessels are shown in Figure 5a

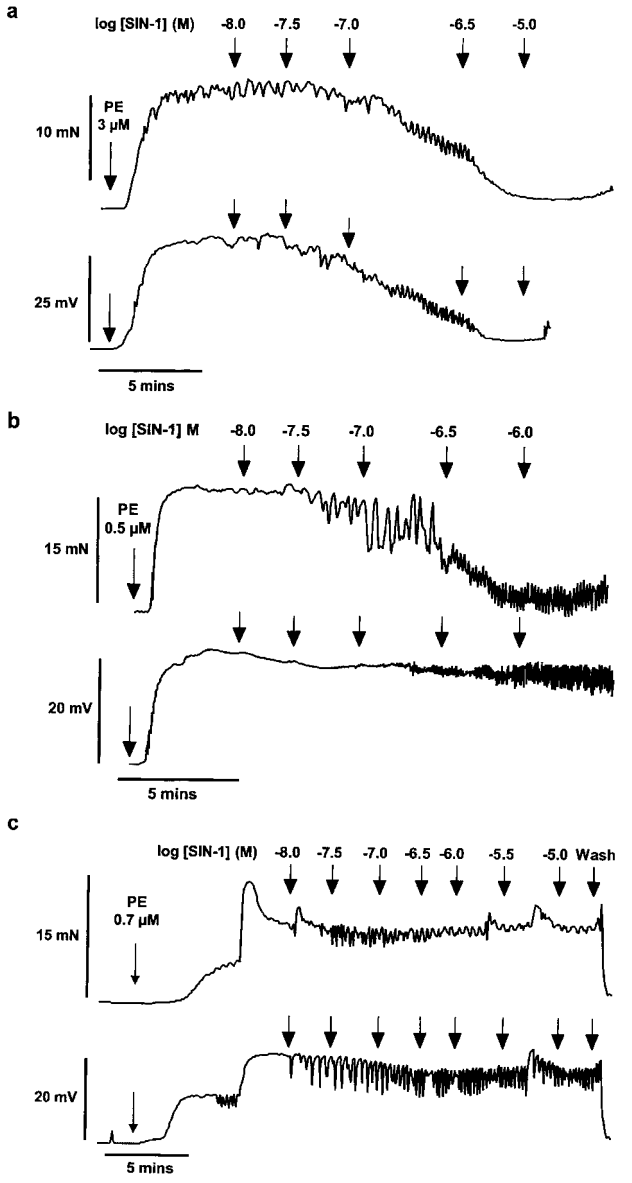

Figure 3 (a) Representative traces illustrating simultaneous changes in tension (mN) and membrane potential (mV) to cumulative additions of SIN-1 (0.01 – 10 μM) in an endothelium-intact arterial segment. (b) Representative traces illustrating simultaneous recordings of changes in tension and membrane potential to cumulative additions of SIN-1 (0.01 – 10 μM) in an endothelium-denuded arterial segment in the presence of ChTX alone and (c) in combination with ODQ.

In endothelium-denuded arteries, IbTX, either in the presence or absence of ODQ, did not significantly inhibit relaxation to SIN-1 ($n=4$ in each case). In contrast, although application of 4-AP alone did not alter relaxations to SIN-1 in endothelium-denuded tissues ($n=6$), in the presence of ODQ, exposure to 4-AP did significantly inhibit relaxation to the NO donor, reducing the maximal response by around 45% to $51\pm2\%$ ($n=6$; $P<0.01$). Furthermore, in the

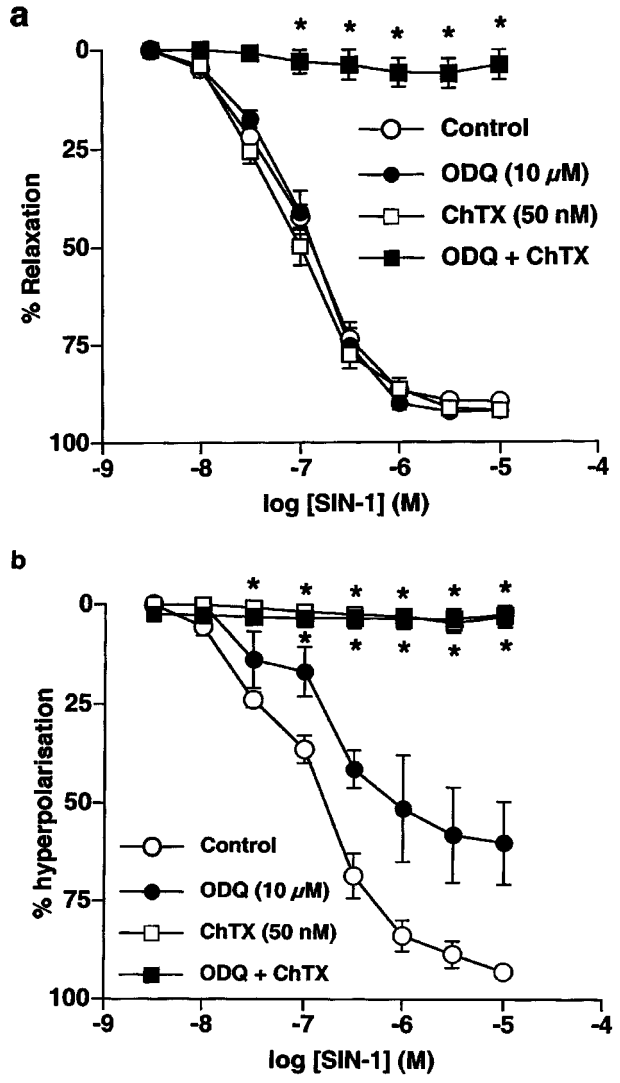

Figure 4 Mean concentration-response curves for SIN-1-evoked relaxation (a) and hyperpolarization (b) in endothelium-denuded arteries in the absence and presence of ODQ (10 μM) and ChTX (50 nM) alone and in combination. All points are the mean of 4–5 observations with s.e.means shown by vertical lines. $^*P<0.01$ compared to control values.

continued presence of ODQ, addition of both 4-AP and IbTX caused a further attenuation of relaxation reducing the maximal relaxation to SIN-1 by around 75% to $24\pm4.5\%$ ($n=6$; $P<0.01$). Mean concentration-response curves showing the effect of IbTX and 4-AP, alone and in combination, on relaxation to SIN-1 in denuded arteries are shown in Figure 5b.

Discussion

The major novel observations in the present study are that smooth muscle hyperpolarization contributes to relaxation with both authentic NO and SIN-1 in the rat isolated small mesenteric artery and that both BK_{Ca} and K_{DR} channels may play a role in this response. The hyperpolarization appears to be independent of cyclic GMP, and presumably involves a direct activation of the potassium channels. These data also indicate that the contribution of this pathway depends on the source of NO. In the case of SIN-1, the mechanism driving relaxation can be modified by the endothelium.

A previous study in the rat mesenteric artery, showed that authentic NO (0.1–1 μmoles) could stimulate a glibenclamide-sensitive hyperpolarization of the smooth muscle cell resting membrane potential. However, when the arteries were depolarized and contracted with noradrenaline, no change in membrane potential was observed (Garland & McPherson, 1992). Our data indicate that although low doses of authentic NO evoke relaxation of rat isolated mesenteric arteries through the formation of cyclic GMP with no change in membrane potential, higher doses of NO (10–150 μM) can cause significant smooth muscle hyperpolarization in phenylephrine-stimulated arteries. This change in membrane potential can make a significant contribution to relaxation to high doses of NO. The change was blocked with ChTX but not with ODQ, indicating a direct cyclic GMP-independent activation of ChTX-sensitive potassium channels.

A similar relationship to concentration of authentic NO and membrane hyperpolarization has been reported in other vessels such as rabbit femoral and guinea-pig uterine arteries (Tare *et al*., 1990; Plane *et al*., 1995). In contrast, in the rabbit carotid artery relaxation to all concentrations of NO was associated with smooth muscle hyperpolarization (Plane *et al*., 1998). At the other extreme, NO failed to evoke a significant change in the smooth muscle membrane potential of either stimulated or unstimulated segments of the rabbit basilar artery, even with concentrations approaching 150 μM (Plane & Garland, 1993). These observations may reflect a wide variation in the sensitivity of target potassium channels to NO, or in the mechanism available for contraction in these different vessels. In smaller arteries, such as the mesenteric, agonist-induced contraction is known to depend largely upon membrane depolarization and the entry of extracellular calcium through voltage-dependent calcium channels (Nilsson, 1998). Thus, relaxation may reflect a greater influence of hyperpolarization on calcium entry through voltage-sensitive channels, compared with other arteries where voltage-independent mechanisms predominate. Also, ChTX-sensitive channels are voltage-dependent, open probability increasing with depolarization. Therefore, in vessels such as the mesenteric artery where contraction is closely linked to smooth muscle membrane potential, agonist-induced depolarization would be expected to increase the open probability of ChTX-sensitive channels and presumably therefore enhance their sensitivity to modulation by NO.

In contrast to the dose-dependent effects of authentic NO on smooth muscle membrane potential, relaxation in the rat mesenteric artery to all concentrations of the NO donor SIN-1 were accompanied by smooth muscle hyperpolarization. Changes in membrane potential to SIN-1 were not modified by the soluble guanylyl cyclase inhibitor, ODQ, but were inhibited with ChTX. This indicates that, as with authentic NO, hyperpolarization to SIN-1 may involve a direct activation of ChTX-sensitive potassium channels.

ChTX can inhibit large-conductance calcium-activated potassium channels (B_{KCa}) and thus these findings extend and lend a functional significance to our recent observations

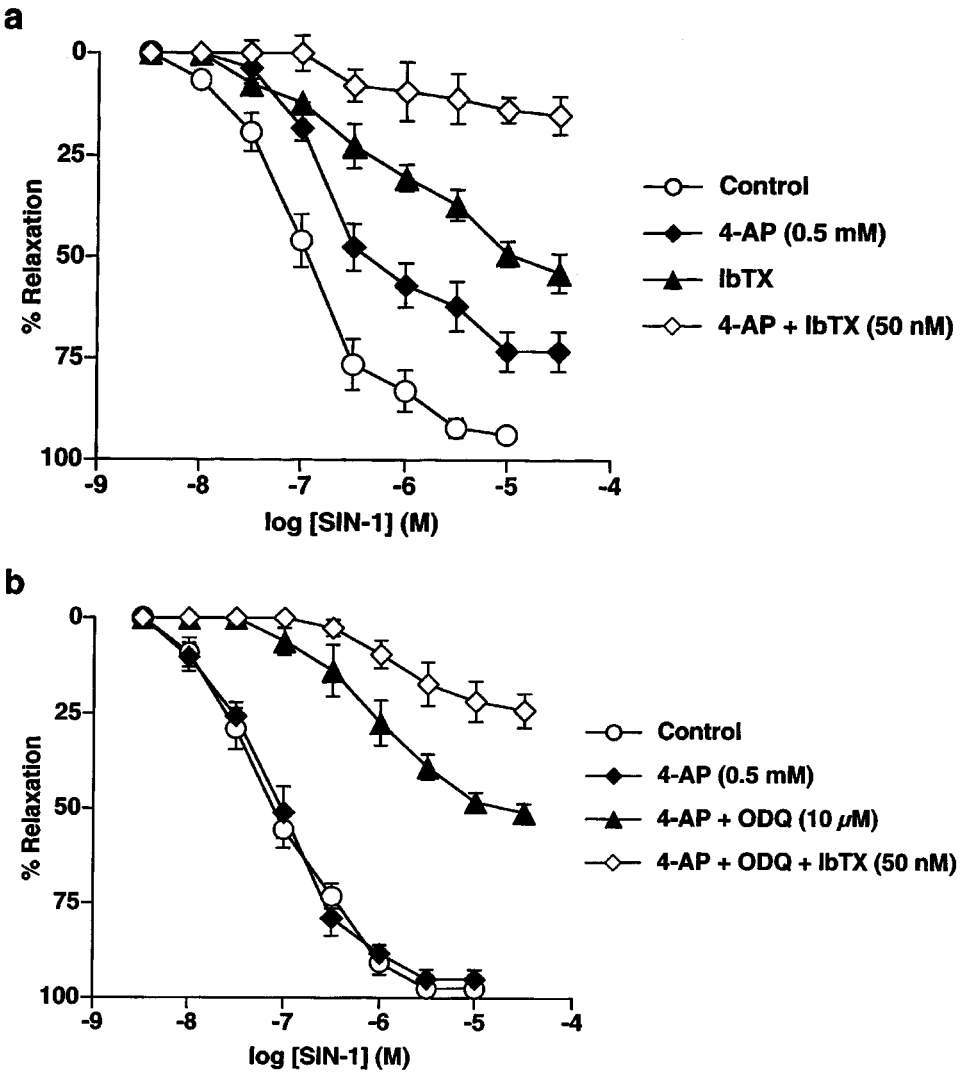

Figure 5 Mean concentration-response curves for SIN-1-evoked relaxation in (a) endothelium intact arteries where relaxation was abolished by either 4-AP (0.5 mM) or IbTX (50 nM) alone, an action which was additive. (b) In denuded arteries, 4-AP was without effect unless it was combined with ODQ or ODQ and IbTX. All points are the means of six observations with the s.e.mean shown by the vertical lines.

on single smooth muscle cells from the same artery, where authentic NO and SIN-1 both appeared to activate ChTX-sensitive B_{KCa} channels independently of cyclic GMP formation (Mistry & Garland, 1998). How this activation occurs is not clear, but NO, or a reactive intermediate, has been suggested to increase B_{KCa} channel activity by nitrosylating sulphydryl groups on the channels or a closely associated protein (Bolotina *et al*., 1994; Ahern *et al*., 1999; Lang *et al*., 2000). So a variation in the ability of NO to activate potassium channels in different arteries may reflect the expression of a range of channel sub-units or of accessory proteins.

In addition to B_{KCa}, ChTX can also inhibit both intermediate conductance calcium-activated potassium channels (I_{KCa}; Brugnara *et al*., 1995) and voltage-sensitive, delayed rectifier potassium channels (K_{DR}; Kaczorowski *et al*., 1996). Thus, the fact that relaxation to SIN-1 was less sensitive to IbTX, a selective inhibitor of B_{KCa}, than to ChTX, may indicate a role for other potassium channels in the response to NO. To date, I_{KCa} has only been reported in proliferating vascular smooth muscle cells and a role for these channels in contractile cells has yet to be demonstrated (Neylon *et al*., 1999). However, I_{KCa} may well be present on endothelial cells (Edwards *et al*., 1998). This localization may help to explain the endothelium-dependent component to the action of SIN-1.

Previous studies have shown that 4-AP, can reduce NO-induced dilatation in pulmonary and umbilical arteries, and that cyclic GMP-independent activation of K_{DR} channels may contribute to NO-evoked responses in these vessels (Zhao *et al*., 1997; Lovren & Triggle, 2000). In the present study, application of 4-AP alone, at a concentration selective for inhibition of K_{DR}, attenuated relaxations to SIN-1 in endothelium-intact strips but was without effect in denuded tissues. However, in both endothelium-intact and denuded arteries, the combination of 4-AP and IbTX caused a similar level of inhibition of SIN-1-evoked relaxations to that observed with ChTX. These data indicate that the inhibitory

effects of ChTX on relaxation to NO in mesenteric arteries may be due to an action on both B_{KCa} and K_{DR} channels. Furthermore, in endothelium-denuded tissues, the inhibitory effects of 4-AP and IbTX were observed in the presence of ODQ indicating that the stimulatory effect of NO on these channels may occur *via* a direct, cyclic GMP-independent mechanism.

The molecular identity of the channels that contribute to K_{DR} currents in mesenteric artery smooth muscle cells is unclear. The pore-forming sub-unit Kv1.5 is widely expressed in vascular tissue, including mesenteric arteries (Xu *et al*., 1999), and is thought to contribute to K_{DR} currents in vascular smooth muscle cells isolated from a number of vessels (Overturf *et al*., 1994; Clément-Chomienne *et al*., 1999). Although sensitive to low concentrations of 4-AP (IC_{50} around 200 μM; Overturf *et al*., 1994; Clément-Chomienne *et al*., 1999), Kv1.5 homomultimeric channels, are resistant to inhibition by ChTX and the presence of one Kv1.5 sub-unit can confer resistance to the toxin on heteromultimeric channels (Russell *et al*., 1994). However, there is now evidence for the expression of other ChTX-sensitive pore-forming sub-units in vascular smooth muscle cells and, although the ChTX-sensitivity of K_{DR} currents in rat mesenteric artery smooth muscle cells has yet to be investigated, expression of both Kv1.2 and Kv1.3 channel proteins in smooth muscle cells from this artery has recently been demonstrated (Xu *et al*., 1999).

In the rabbit carotid artery, the ability of authentic NO and SIN-1 to cause hyperpolarization and relaxation was similar in both endothelium-intact and denuded arteries (Plane *et al*., 1998). This is consistent with a number of other studies, predominantly involving larger arteries, in which no differences were reported between the relaxant actions of authentic NO in intact and denuded preparations (Huang *et al*., 1988; Tare *et al*., 1990). In contrast, the present data from the rat mesenteric artery reveal that a functional endothelium in some way decreased the ability of authentic NO to evoke hyperpolarization and relaxation. This characteristic was not shared with SIN-1, which induced responses in intact and denuded vessels with equal potency. However, the importance of the change in membrane potential to the relaxation induced with SIN-1 could be modulated by the endothelium. In endothelium-intact segments, the hyperpolarization underpinned relaxation, whereas in endothelium-denuded arteries full relaxation could still be achieved without a change in membrane-potential. In the rabbit mesenteric artery, endothelium-dependent modulation has also been described with NO-induced responses. Smooth muscle hyperpolarization to either SIN-1 or sodium nitroprusside was only observed in the absence of the endothelium or if the generation of endothelium-derived NO was inhibited (Murphy & Brayden, 1995). The authors suggested that this might reflect a desensitization of the hyperpolarizing response by the endogenous release of NO.

Our data suggest this is not the case in rat mesenteric arteries. In endothelium-intact vessels, relaxation to SIN-1 can be almost totally accounted for by cyclic GMP-independent, ChTX-sensitive hyperpolarization. Thus, it appears that an intact endothelial cell layer in some way reduces the ability of SIN-1 to stimulate relaxation *via* the membrane potential-independent, cyclic GMP-mediated pathway. Previous studies have shown that the basal release of endothelium-derived NO depresses the sensitivity of soluble guanylyl cyclase to the nitrovasodilators, sodium nitroprusside and SIN-1 (Busse *et al*., 1989; Moncada *et al*., 1991; Brandes *et al*., 2000). This effect can be reversed by acute inhibition of NO synthase (Brandes *et al*., 2000). However, our previous studies have demonstrated that SIN-1's ability to increase cyclic GMP is not significantly different in the presence or absence of basal NO release (Plane *et al*., 1996). This suggests that the endothelium-dependent modulation of the relaxation pathway occurs downstream of the formation of cyclic GMP, possibly at the level of the phosphodiesterases.

In conclusion, in rat small mesenteric arteries both authentic NO and SIN-1 can evoke smooth muscle hyperpolarization and relaxation, but with different characteristics. Authentic NO did evoke ChTX-sensitive smooth muscle hyperpolarization, but only in high concentrations. This was in contrast to the glibenclamide-sensitive hyperpolarization of the resting membrane potential in this artery evoked with low concentrations of NO (Garland & McPherson, 1992). However, SIN-1 evoked smooth muscle hyperpolarization in the same concentration-range as relaxation, and in the presence of an intact endothelium the change in membrane potential was the primary drive to relaxation. These differences in the response characteristics between authentic NO and NO generated from SIN-1 may reflect the action of additional breakdown products from SIN-1 (Feelisch & Stamler, 1996). As such, they emphasise the importance of using authentic NO in any investigation of the mechanisms responsible for smooth muscle relaxation.

This work was supported by a Programme Grant from the Wellcome Trust. L.J. Sampson was supported by an MRC studentship. F. Plane is currently a Wellcome Career Development Fellow.

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(Received March 19, 2001
Revised April 20, 2001
Accepted April 20, 2001)