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A comparative study on the rat aorta and mesenteric arterial bed of the possible role of nitric oxide in the desensitization of the vasoconstrictor response to an α_1-adrenoceptor agonist

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1 In thoracic aortic strips with intact endothelium, the first and second (1 h later) dose-response curves obtained with methoxamine were almost the same.

2 Methoxamine caused a dose-dependent increase in perfusion pressure in the rat isolated mesenteric arterial bed, but the second (1 h later) dose-response curve for methoxamine showed a significant attenuation of the response in comparison with the first.

3 The attenuation shown by the second dose-response curve for methoxamine was significantly reduced, but not abolished, in mesenteric arterial beds without endothelium. Incubating endothelium-intact mesenteric arterial beds with N^G-nitro-L-arginine (L-NOARG) caused a significant, but not complete, reversal of the attenuation shown in the second dose-response curve.

4 Incubating the mesenteric arterial bed with capsaicin, tetrodotoxin, indomethacin or with isotonic high k^+ (60 mM) plus nicardipine did not affect the above attenuation seen in the second dose-response curve.

5 The guanosine 3′:5′-cyclic monophosphate (cyclic GMP) level in the effluent from the perfused mesenteric arterial bed was significantly increased after the second exposure to methoxamine. This effect was significantly smaller after removal of the endothelium or pretreatment with L-NOARG.

6 These results suggest that a desensitization to methoxamine develops rapidly in the mesenteric arterial bed, but not in the aorta, and that release of nitric oxide from the endothelium plays a major role in this desensitization.

Keywords: Desensitization; mesenteric arterial bed; methoxamine; endothelium; cyclic GMP

Introduction

Prolonged exposure or repetitive application of α-agonists of KCl, or repeated tension loading results in desensitization: a decreased ability of the tissue to respond to subsequent stimulation by the same agonists (Lurie *et al*., 1985; Nakaki *et al*., 1990; Hiremath *et al*., 1991; Sunano *et al*., 1991; Kaneko & Sunano, 1993; Hu *et al*., 1994). It has been shown that the contractile responses of some blood vessels to α-adrenoceptor agonists are regulated by the endothelium (Cocks & Angus, 1983; Egleme *et al*., 1984; Carrier & White, 1985; Martin *et al*., 1986; Macleod *et al*., 1987; Cohen *et al*., 1988; Amerini *et al*., 1995). Interestingly, the endothelium-dependent depression of noradrenaline-induced contractions is more marked when the contraction is evoked repeatedly (Nakaki *et al*., 1990; Sunano *et al*., 1991; Kaneko & Sunano, 1993).

Much of the research on the effects of prolonged exposure or repetitive application of α-agonists has focused on the rat aorta. Indeed, few studies of this type have been made on the contractile response of smaller vessels such as those of the rat mesenteric arterial bed. This omission is regrettable because, as the mesenteric circulation of the rat receives approximately one fifth of the total cardiac output (Nichols *et al*., 1985), its regulation may have a considerable impact on systemic blood pressure and circulating blood volume. It would not be surprising if the mesenteric arterial bed were to behave differently to the aorta. Indeed, there is much experimental evidence to indicate that the relative response to a given stimulus (either physiological or pharmacological) depends on the position and nature of the vessel investigated (Delashaw & Duling, 1991; Kurz *et al*., 1991). Thus, one can differentiate between large conductive vessels and small resistance vessels. Moreover, in most vascular networks, vascular reactivity changes dramatically at the transition from conductive to resistance segments (Edvinsson *et al*., 1989; Kurz *et al*., 1991). This applies to the responses to humoral factors and to metabolic mediators such as endothelium-derived relaxing factor (EDRF) or nitric oxide (NO) (Griffith *et al*., 1987; Dhein *et al*., 1995).

The relative insensitivty of endothelium-dependent effects to guanylate cyclase inhibitors in the rat mesenteric artery (Furchgott *et al*., 1987) raises the possibility that this effect may be mediated via another mechanism, possibly increased potassium permeability (Bolton *et al*., 1984; Komori & Suzuki, 1987; Feletou & Vanhoutte, 1988; Garland & McPherson, 1992). Indeed, we recently found that acetylcholine (ACh) releases NO as well as endothelium-derived hyperpolarizing factor (EDHF) in the rat mesenteric arterial bed (Kamata *et al*., 1995; 1996).

When we began to investigate the involvement of EDRF in the desensitization of blood vessels to α-agonists, we found unexpectedly that there was a marked decline in the concentration-dependent constriction of the mesenteric arterial bed in the second series of applications of methoxamine in comparison with the response in the first series. We therefore decided to examine this desensitization. We used methoxamine as the α-agonist, because this drug does not undergo uptake into sympathetic nerve endings. The present study was designed to characterize α_1-adrenoceptor-mediated desensitization in the rat aorta and mesenteric arterial bed, and to investigate the mechanism responsible for the desensitization.

Methods

Male Wistar rats aged 8 weeks were housed under controlled conditions (temperature 21–22°C, relative air humidity 50±5%). Food and water were available *ad libitum* to all animals. This study was conducted in accordance with the

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Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (which is accredited by the Ministry of Education, Science, Sports and Culture, Japan).

Preparation of the mesenteric arterial bed

Male Wistar rats, weighing from 250 to 350 g, were anaesthetized with ether and then given an intravenous injection of 1000 units kg^{-1} of heparin. Following the injection, a midline incision was made, and the mesenteric arterial bed rapidly dissected out and placed into modified Krebs-Henseleit solution (KHS, composition in mM: NaCl 118.0, KCl 4.7, $NaHCO_3$ 25.0, $CaCl_2$ 1.8, NaH_2PO_4 1.2, $MgSO_4$ 1.2, dextrose 11.0 and 0.25% bovine serum albumin). The mesenteric artery and vein were tied off near the caecum, and the remaining intestine was then separated from the arterial bed along the intestinal wall. The mesenteric arterial bed was perfused by the method described by McGregor (1965), with various modifications previously described by us (Kamata *et al*., 989; Abiru *et al*., 1993). Briefly, warm (37°C), oxygenated (95% O_2 – 5%CO_2) KHS was pumped into the mesenteric arterial bed, by a peristaltic pump operating at a rate of 5 ml min^{-1}, through a cannula inserted into the superior mesenteric artery. Vascular responses were detected as changes in perfusion pressure, which was monitored continuously with a pressure transducer (Nihon Kohden, Model AP2001, Tokyo, Japan) and recorded on a pen recorder. Following a 60 min equilibrium period, the perfusion circuit was transformed into a closed system by collecting the perfusate in a second bath and from thence recirculating it through the mesenteric arterial bed. The total volume of the closed system was 50 ml, and agents were administered via the bath. In some preliminary experiments, the mesentery preparation was constricted by perfusion with a solution containing 5×10^{-6} to 4×10^{-5} M methoxamine, which resulted in a perfusion pressure of approximately 115–130 mmHg and then maximally relaxed with a perfusion solution containing 10^{-6} M ACh, a response which confirmed the integrity of the endothelium in our preparation. Dose-response curves for methoxamine (10^{-7} to 3×10^{-4} M) and for U-46619 (10^{-9} to 10^{-5} M) were obtained by cumulatively increasing the total concentration of the agonist in the bath. One hour after the first dose-response curve had been obtained for methoxamine or U-46619, the same agent was again administered cumulatively. When agonist-induced changes in perfusion pressure in the mesenteric arterial bed were to be examined, we did not test the ACh-induce vasodilatation, to avoid desensitization to agonists. To investigate the influence of 10^{-4} M N^G-nitro-L-arginine (L-NOARG), isotonic high K^+ (60 mM), 10^{-5} M indomethacin, 10^{-5} M capsaicin and 10^{-6} M tetrodotoxin on the agonist-induced contractile responses in the mesenteric arterial bed, the mesentery was incubated for 30 min in the appropriate medium before the cumulative addition of the agonist. To exclude the involvement of EDHF in the methoxamine-induced increase in perfusion pressure, some mesenteric preparations were depolarized with isotonic high K^+ (60 mM) in the presence of nicardipine (10^{-7} M) before being constricted. Each antagonist and isotonic high K^+ medium was tested on a different preparation. In some experiments, the mesenteric preparation was perfused with acetic Trito X-100 for 1 min to remove functionally the endothelial cells lining the resistance vessels. This treatment reduced the >90% the relaxation that had previously been induced by the maximally effective dose of ACh (10^{-6} M) without reducing the contractile effects of methoxamine.

Preparation of thoracic aortic strips

A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified KHS. The aorta was cleaned of loosely adhering fat and connective tissue and cut into helical strips 3 mm in width and 20 mm in length. The tissue was placed in well-oxygenated (95% O_2-5% CO_2) KHS at 37°C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden TB-611T, Tokyo, Japan). It was then allowed to equilibriate for 60 min at a resting tension of 1.0 g. During this time, the KHS in the tissue bath was replaced every 20 min.

After equilibration, each aortic strip was contracted by treatment with 10^{-7} M noradrenaline (NA). The presence of functional endothelial cells was confirmed by demonstrating the ability of the strips to relax to 10^{-5} M ACh and aortic strips in which an 85% relaxation of the NA response occurred

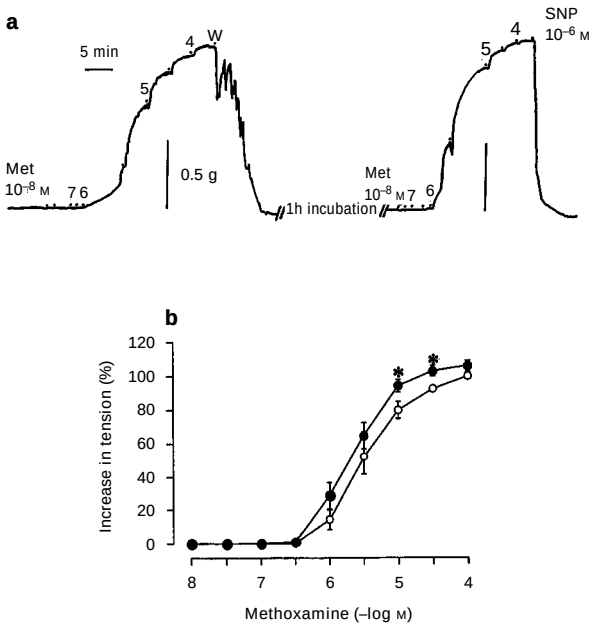

Figure 1 Dose-dependent response of aortic strips to methoxamine. (a) Typical records showing the contractile response of the aorta to cumulative application of methoxamine (10^{-8} to 10^{-4} M). After a wash (W), the preparation was incubated in normal medium for 1 h, then tested again with methoxamine as before. At the end of the experiment, the ability of sodium nitroprusside (SNP, 10^{-6} M) to induce maximal relaxation was confirmed. The dots indicate when drug was added. The single numerals paired with the dots indicate the cumulative concentration of the drug (e.g., 7 refers to 10^{-7} M). The data are summarized in (b): first application ($n=6$, ○), second application ($n=6$, ●). *$P<0.05$ for comparison with first dose-response curve. Ordinate scale shows increase in tension expressed as a percentage of the peak response to the first application of methoxamine.

Table 1 Maximal contraction and EC_{50} values for methoxamine-induced contraction of aortic strips

	First application		*Second application*	
Treatments	*Max. cont.* (%)	*log EC_{50}* (M)	*Max. cont.* (%)	*log EC_{50}* (M)
Control	100	−5.5±0.1	115.6±6.1*	−5.8±0.1
−EC	100	−5.6±0.1	89.9±19.6	−5.7±0.1
L-NOARG	100	−5.5±0.1	106.2±3.4	−5.6±0.1

Values are mean±s.e.; $n=6$ animals. *Statistically different from the first application ($P<0.05$). −EC, without endothelium; L-NOARG, N^G-nitro-L-arginine (10^{-4} M).

were regarded as tissues with endothelium. The removal of endothelial cells in some strips by rubbing was confirmed by the loss of the ACh-induced relaxation. To examine the effect of indomethacin, tetrodotoxin, N^G-nitro-L-arginine (L-NOARG) and capsaicin on methoxamine responses, the aortic strips were exposed to these agents for 30 min.

Measurement of cyclic GMP

The guanosine 3′:5′-cyclic monophosphate (cyclic GMP) content of the perfusate was assayed as previously described by Kamata *et al.* (1996). Briefly, the mesenteric preparation was perfused with KHS containing 3-isobutyl-methylxanthine (IBMX, 10^{-4} M) to inhibit phosphodiesterase activity. For the cyclic GMP determination, the perfusate was collected on four occasions, each over a 30 s period, as follows: sample-1, before application of methoxamine; sample-2, after increasing concentrations of methoxamine had been applied and the methoxamine-induced contractile response had stabilized; sample-3, after the mesentery had been washed with KHS and incubated with KHS for one hour, but before the second application of methoxamine; sample-4, after increasing concentrations of methoxamine had been applied for the second time and the methoxamine-induced contractile response had stabilized. The samples were stored at −20°C until the concentration of cyclic GMP was to be determined by radioimmunoassay with a commercially available kit (Yamasa Cyclic GMP Assay Kit, Yamasa Corp., Choshi, Japan). The recovery rate of the cyclic GMP content in each column was calculated by counting the radioactivity due to [^{3}H]-cyclic GMP with a liquid scintillation counter (Aloka, Tokyo, Japan), and the values for cyclic GMP content obtained from the radioimmunoassay were corrected for with this recovery rate. The recovery rate for the cyclic GMP content in each column was within the range 85 to 95%. The release of cyclic GMP induced by methoxamine is expressed as fmol 100 μl^{-1}.

Drugs

Methoxamine hydrochloride, N^G-nitro-L-arginine (L-NOARG), indomethacin, tetrodotoxin, capsaicin, bovine serum albumin (Fraction V), Triton X-100 and 3-isobutyl-methylxanthine (IBMX) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Of the other agents, 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U-46619) was purchased from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.), and acetylcholine chloride was purchased from Daiichi (Tokyo, Japan).

Statistics

Data are expressed as the means ± s.e.mean. Statistical differences were assayed by Student's *t* test for unpaired observations, following a one-way analysis of variance.

Results

Contractile responses of aortic strips and mesenteric arterial beds to methoxamine

Cumulative application of methoxamine (10^{-8} to 10^{-4} M) caused contraction of the aortic strips with intact endothelium in a dose-dependent manner (Figure 1 and Table 1). In these strips, the second dose-response curve for methoxamine was almost the same as the first. In marked contrast, when we looked at the increase in perfusion pressure in the mesenteric arterial bed induced by methoxamine (10^{-7} to

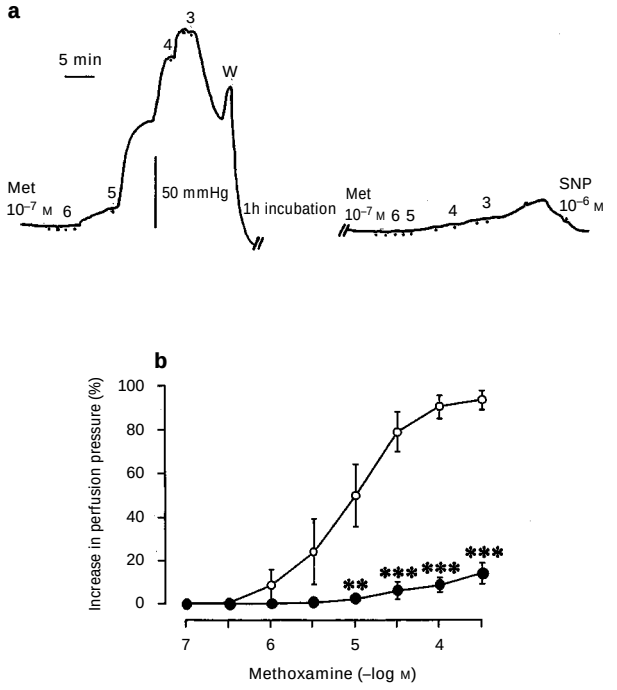

Figure 2 Concentration-dependent increase in perfusion pressure in the mesenteric arterial bed in response to methoxamine. (a) Typical records showing the response of the bed to cumulative application of methoxamine (10^{-7} to 3×10^{-4} M). After a wash (W), the mesenteric preparation was incubated with normal medium for 1 h, then tested again with methoxamine as before. At the end of the experiment, the ability of sodium nitroprusside (SNP, 10^{-6} M) to induce maximal relaxation was confirmed. The dots indicate when drug was added. The single numerals paired with the dots indicate the cumulative concentration of the drug (e.g., 6 refers to 10^{-6} M). The data are summarized in (b): first application ($n=6$, ○), second application ($n=6$, ●). $^{**}P<0.01$, $^{***}P<0.001$ for comparison with first dose-response curve. Ordinate scale shows increase in tension expressed as a percentage of the peak response to the first application of methoxamine.

Table 2 Maximal increase in perfusion pressure and EC_{50} values for methoxamine-induced increase in perfusion pressure

	First application		*Second application*	
Treatments	*Max. increase* (%)	*log EC_{50}* (M)	*Max. increase* (%)	*log EC_{50}* (M)
Control (methoxamine)	96.1 ± 3.9	−5.1 ± 0.2	22.7 ± 8.0***	−4.1 ± 0.2*
−EC	100	−5.7 ± 0.1	77.5 ± 4.2***	−5.1 ± 0.1***
L-NOARG	100	−5.1 ± 0.1	96.1 ± 3.9	−4.7 ± 0.3
Indomethacin	100	−4.7 ± 0.1	13.3 ± 5.0***	−4.3 ± 0.1*
High K^+	100	−4.7 ± 0.1	23.6 ± 8.8***	−4.6 ± 0.7
Capsaicin	95.5 ± 3.4	−4.9 ± 0.1	17.1 ± 6.7***	−4.3 ± 0.1**
TTX	100	−5.0 ± 0.1	14.2 ± 5.7***	−4.4 ± 0.1***
Control (U-46619)	100	−6.5 ± 0.1	84.2 ± 11.7	−6.1 ± 0.2*
−EC	100	−7.01 ± 0.2	116.7 ± 6.8	−7.0 ± 0.1

Values are mean ± s.e.; $n=6$ animals. −EC, without endothelium; L-NOARG, N^G-nitro-L-arginine (10^{-4} M); indomethacin (10^{-5} M); high K^+, isotonic high K^+ (60 mM) in the presence of 10^{-7} M nicardipine; capsaicin (10^{-5} M); TTX, tetrodotoxin (10^{-6} M). Significantly different from the first application. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

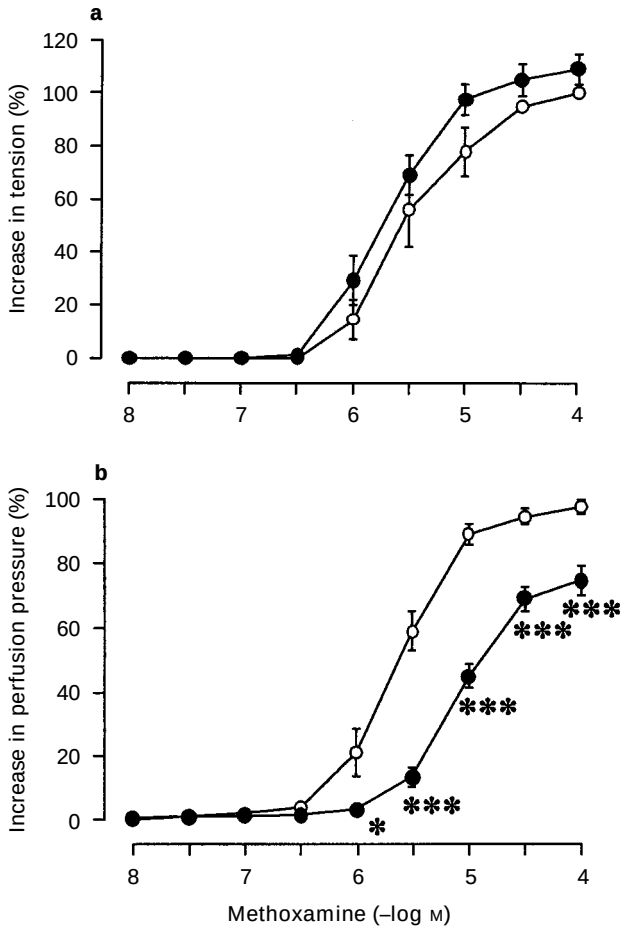

Figure 3 First and second dose-response curves for the effect of methoxamine on the endothelium-denuded aorta and mesenteric arterial bed; (a) aorta; (b) mesenteric arterial bed. First application ($n=6$, ○), second application ($n=6$, ●). $^{*}P<0.05$, $^{***}P<0.001$ for comparison with first dose-response curve. Ordinate scale shows increase in tension expressed as a percentage of the peak response to the first application of methoxamine.

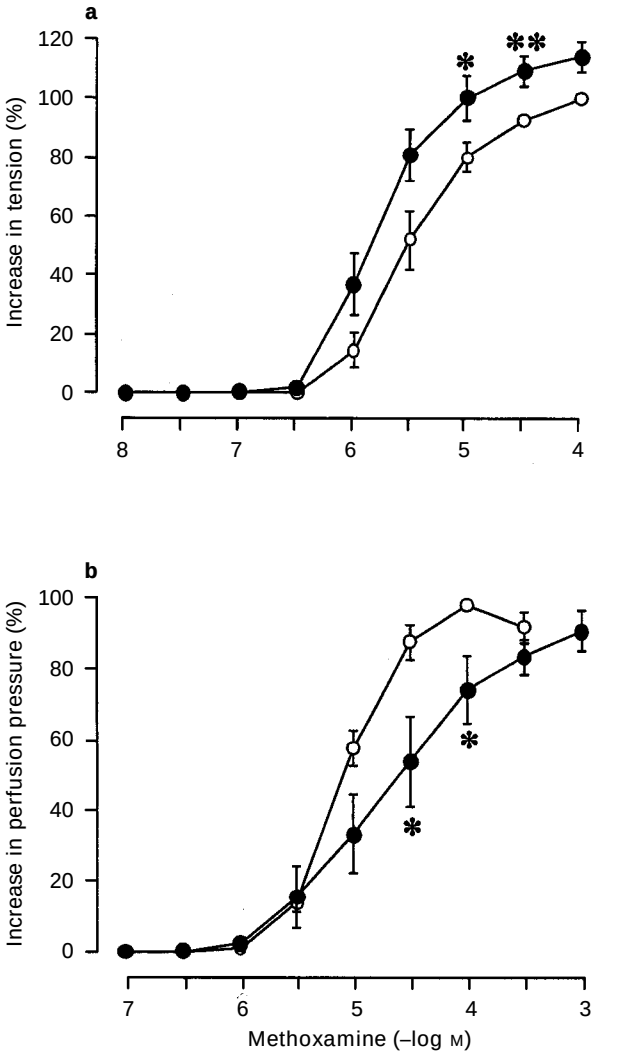

Figure 4 First and second dose-response curves for the effect of methoxamine on the aorta and mesenteric arterial bed in preparations pretreated with L-NOARG (10^{-4} M); (a) aorta; (b) mesenteric arterial bed. First application ($n=6$, ○), second application ($n=6$, ●). $^{*}P<0.05$, $^{**}P<0.01$ for comparison with first dose-response curve. Ordinate scale shows increase in tension expressed as a percentage of the peak response to the first application of methoxamine.

3×10^{-4} M), we found that the second dose-response curve showed a greatly decreased response in comparison with the first (Figure 2 and Table 2). EC_{50} values are given in Tables 1 and 2.

In aortic strips without endothelium, there was again no difference between the first and second dose-response curve (Figure 3 and Table 1). When we used mesenteric arterial beds without endothelium, there was significantly less attenuation of the response than there had been in beds with intact endothelium (i.e. the second dose-response curve was closer to the first). Even so, the second dose-response curve was shifted to the right somewhat, and the maximal contraction was lower for the second curve than for the first (Figure 3 and Table 2).

Effects of antagonists and depolarization on the responses in the second series of methoxamine applications

In aortic strips with intact endothelium, L-NOARG (10^{-4} M) elevated slightly but significantly the second dose-response curve for methoxamine (Figure 4 and Table 1). In the endothelium-intact mesenteric arterial bed pretreated with L-NOARG (10^{-4} M) for 30 min, the attenuation of the methoxamine-induced response shown by the second dose-response curve was inhibited, although not completely, i.e. the second dose-response curve was only slightly shifted to the right of the first (Figure 4 and Table 2).

To exclude the involvement of EDHF in the methoxamine-induced increase in perfusion pressure, some mesenteric preparations were depolarized with isotonic high K^{+} (60 mM) solution in the presence of nicardipine (10^{-7} M) before a constriction was induced with methoxamine. In such depolarized preparations, the second dose-response curve again showed a significant depression of the response to methoxamine (Figure 5). Preincubation with indomethacin (10^{-5} M), capsaicin (10^{-5} M) or tetrodotoxin (TTX) (10^{-6} M) did not affect the depression of the response seen in the second dose-response curve in mesenteric preparations (compare Figure 5 and 6 with Figure 2, and see Table 2).

Effect of removal of the endothelium on the U-46619-induced increased in perfusion pressure in the mesenteric preparation

We also examined the difference between the first and second dose-response curves for the U-46619-induced increase in perfusion pressure in the mesenteric arterial bed. U-46619 is a thromboxane A_2-mimetic agent (Coleman *et al.*, 1981). The second dose-response curve for the effect of the presence of U-46619 (10^{-9} to 10^{-5} M) showed a significant attenuation of the

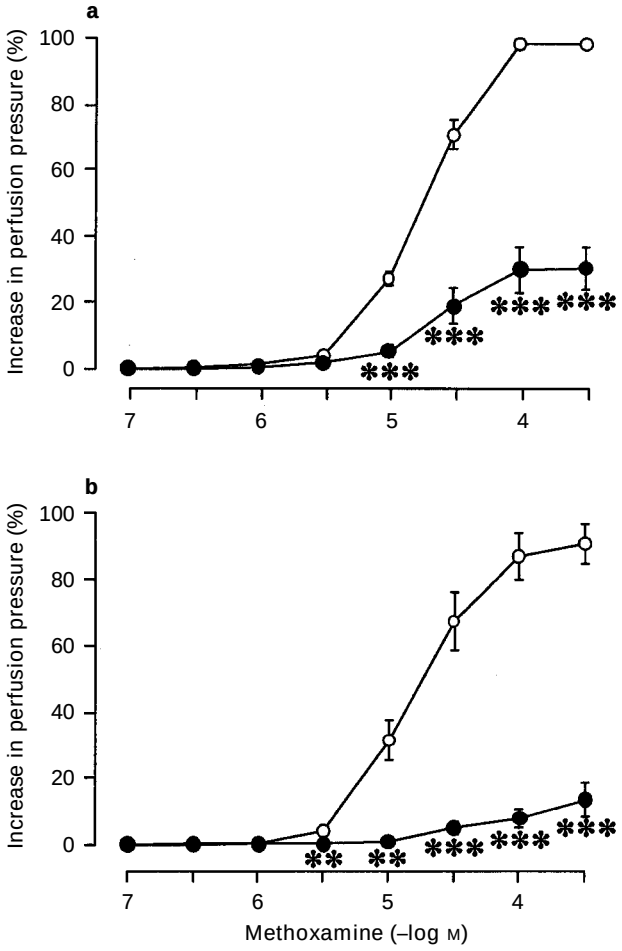

Figure 5 First and second dose-response curves for the effect of methoxamine on the mesenteric arterial bed in preparations pretreated with: (a) isotonic high K^+ (60 mM); (b) indomethacin (10^{-5} M). First application ($n=6$, ○), second application ($n=6$, ●). $^{**}P<0.01$, $^{***}P<0.001$ for comparison with first dose-response curve. Ordinate scale shows increase in tension expressed as a percentage of the peak response to the first application of methoxamine.

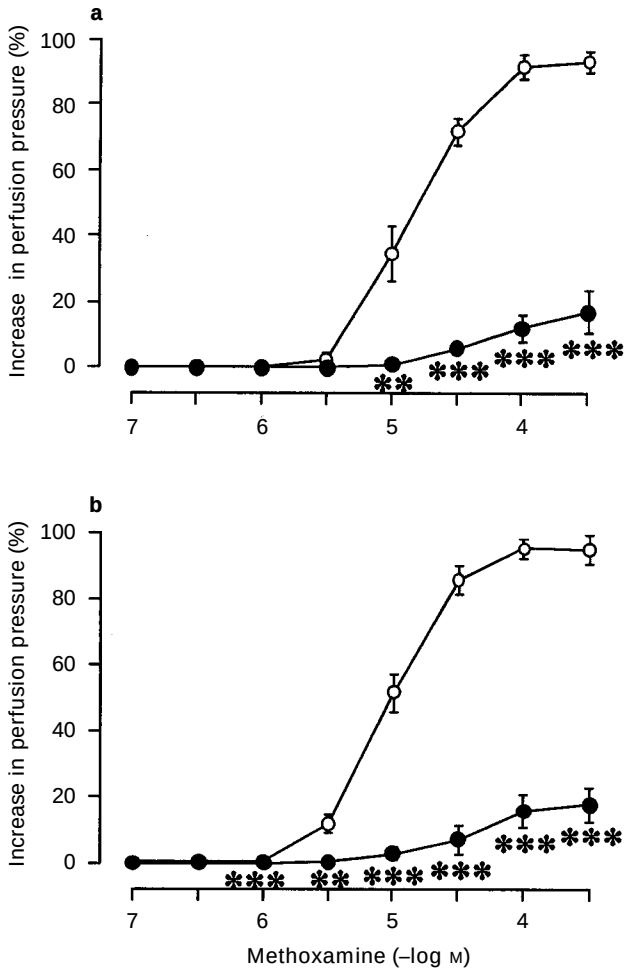

Figure 6 First and second dose-respone curves for the effect of methoxamine on the mesenteric arterial bed in preparations pretreated with: (a) capsaicin (10^{-5} M), (b) tetrodotoxin (10^{-6} M). First application ($n=6$, ○), second application ($n=6$, ●). $^{**}P<0.01$, $^{***}P<0.001$ for comparison with first dose-response curve. Ordinate scale shows increase in tension expressed as a percentage of the peak response to the first application of methoxamine.

response by comparison with the first (Figure 7, Table 2). This attenuation was completely inhibited in endothelium-free preparations (Figure 7).

Effect of methoxamine on the cyclic GMP levels in the perfusate

The basal level of cyclic GMP in the effluent collected from the perfused endothelium-intact mesenteric arterial bed was 20.0 ± 0.6 fmol 100 μl^{-1} ($n=4$). After the first cumulative application of methoxamine (10^{-8} to 10^{-4} M), the level was 24.4 ± 2.3 fmol 100 μl^{-1} ($n=4$), and before the second application of methoxamine it was 29.4 ± 5.0 fmol 100 μl^{-1} ($n=4$). When the second methoxamine (10^{-8} to 10^{-4} M)-induced contraction had stabilized, the cyclic GMP level in the effluents was significantly higher at 61.7 ± 7.3 fmol 100 μl^{-1} ($P<0.05$, after the second vs after the first application, $n=4$) as shown in Figure 8. In mesenteric arterial beds that were either without endothelium or had been pretreated with L-NOARG (10^{-4} M) for 30 min, the methoxamine-induced increase in cyclic GMP was significantly inhibited.

Discussion

The main conclusion from the present study is that a desensitization to the α_1-agonist of methoxamine develops rapidly in the mesenteric arterial bed, but not in the aorta, and that the release of NO from the endothelium plays a major role in this desensitization. In the present study, we found (i) that the second dose-response curve for the methoxamine induced increase in perfusion pressure in the mesenteric arterial bed showed a significant attenuation of the response by comparison with the first, and (ii) that this attenuation was significantly inhibited by the removal of the endothelium or by pretreatment with L-NOARG. The cyclic GMP level in the effluent from the perfused mesentery preparation was significantly increased after the second series of methoxamine applications and this effect was also markedly inhibited by the removal of the endothelium or by pretreatment with L-NOARG.

Vasoactive agents such as ACh cause endothelium-derived relaxation by provoking the release of endothelium-derived relaxing factor (EDRF) in various blood vessels (Furchgott, 1983). Nitric oxide (NO) is a candidate for EDRF (Palmer *et al.*, 1987); it is formed from L-arginine (Palmer *et al.*, 1988; Moncada *et al.*, 1989) and has been shown to stimulate the production of cyclic GMP in smooth muscle cells (Forstermann, 1986; Ignarro *et al.*, 1987). It is also apparent that agonist-mediated contractile responses are modified by the presence or absence of endothelium in many blood vessels (Cocks & Angus, 1983; Egleme *et al.*, 1984; Miller *et al.*, 1984; Lues & Schumann, 1984). Indeed, we confirmed, in the present study, that the contractile response of the aorta to methox-

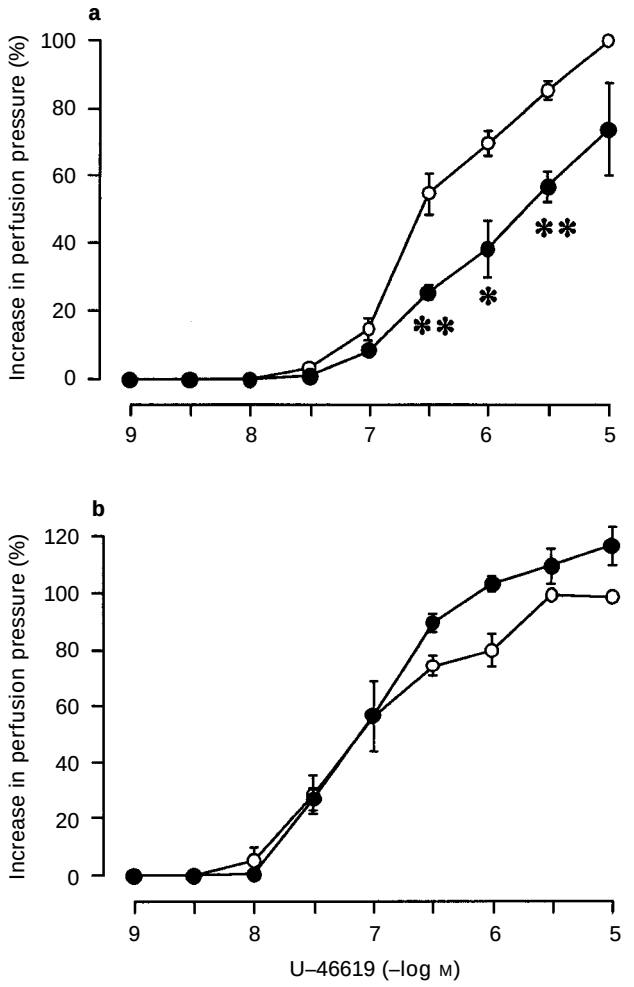

Figure 7 Effect of the removal of the endothelium on the difference between first and second dose-response curves for the effect of U-46619 on the mesenteric arterial bed. (a) Endothelium-intact control, (b) after removal of the endothelium. First application ($n=6$, ○), second application ($n=6$, ●). $^*P<0.05$, $^{**}P<0.01$ for comparison with first dose-response curve. Ordinate scale shows increase in tension expressed as a percentage of the peak response to the first application of methoxamine.

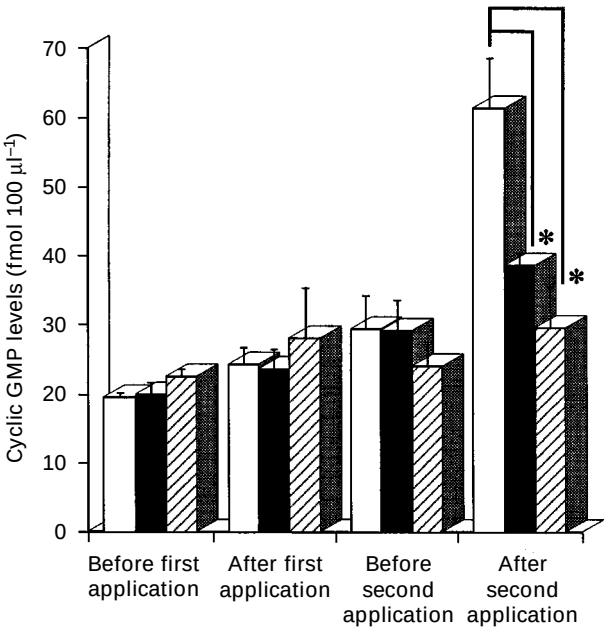

Figure 8 Cyclic GMP levels in effluent from the perfused mesenteric arterial bed. Cyclic GMP levels were measured before and after the first application of methoxamine, and before and after the second application of methoxamine. Control ($n=4$, open columns), without endothelium ($n=4$, solid columns) and after treatment with 10^{-4} M L-NOARG ($n=4$, hatched columns). $^*P<0.05$.

amine, a selective α_1-adrenoceptor agonist, was enhanced by treatment with L-NOARG, an inhibitor of NO synthase (Ishii *et al*., 1990). This indicates that an increase in contractile tone or stimulation of α_1-adrenoceptors on endothelial cells may stimulate them to produce NO, which then counteracts the smooth muscle contraction. The finding that endothelial cells express α_1-adrenoceptors (Sherline & Mascardo, 1984), is consistent with this idea.

After chronic exposure of the thoracic aorta to adrenoceptor agonists, there is a desensitization of α_1-adrenoceptor-mediated contractions (Lurie *et al*., 1985; Nakaki *et al*., 1990; Hiremath *et al*., 1991; Sunano *et al*., 1991; Kaneko & Sunano, 1993; Hu *et al*., 1994). Such an endothelium-dependent depression of noradrenaline-induced contractions is more marked when the contraction is evoked repeatedly (Nakaki *et al*., 1990; Sunano *et al*., 1991; Kaneko & Sunano, 1993). However, in the latter studies, prolonged exposure (6 to 7 h) or repetitive application (5 to 6 times) was needed to induce desensitization (Lurie *et al*., 1985; Hiremath *et al*., 1991; Sunano *et al*., 1991; Kaneko & Sunano, 1993; Hu *et al*., 1994). In marked contrast to these data, we found in the present study that the desensitization to methoxamine in the mesenteric arterial bed was evident on the second application. This desensitization was significantly inhibited by the removal of the endothelium or by pretreatment with L-NOARG (as mentioned above) but not by pretreatment with indomethacin, capsaicin or tetrodotoxin. From these findings, it is most likely that the second application of methoxamine to the mesenteric vascular bed preparation stimulated NO release from the endothelium. This conclusion is supported by the finding that the cyclic GMP level in the effluent from the perfused bed was significantly increased after the second application of methoxamine (but not before), and by the finding that this effect was significantly reduced by the removal of the endothelium or by pretreatment with L-NOARG. In marked contrast, in the aorta, the second series of contractile responses to methoxamine was not different from the first, suggesting that the characteristics of the endothelium may differ significantly between the aorta and the mesenteric arterial bed.

Nakaki *et al*. (1990) have shown that repeated tension-loading induces endothelium-dependent desensitization in the rat aorta. This finding strongly suggests that repeated contraction of the blood vessels stimulates the release of NO, which counteracts the vasoconstriction. In the present study, therefore, we looked for a difference between the first and second dose-responses curve for the U-46619 induced increase in perfusion pressure in the mesenteric arterial bed. U-46619 is a thromboxane A_2-mimetic agent (Coleman *et al*., 1981). In the endothelium-intact mesenteric arterial bed, the U46619-induced second dose-response curve showed a significant attenuation of the response by comparison with the first. By contrast, in the mesenteric arterial bed without endothelium, there was no difference between the first and second dose-response curves. These results suggest that contractile agents may non-specifically induce endothelium-dependent desensitization in the mesenteric arterial bed.

Neither the removal of the endothelium nor treatment with L-NOARG completely reversed the methoxamine-induced desensitization. The component of the loss in sensitivity to methoxamine that persisted in the endothelium-free mesenteric preparation presumably reflects changes at the level of the vascular smooth muscle itself. Indeed, it has been found that prolonged activation of α-adrenoceptors in vascular smooth muscle leads to down-regulation of protein kinase (Hu *et al*., 1992). However, these changes are relatively small compared

to the desensitization seen in the presence of an intact endothelium. It is most likely, therefore, that the endothelium plays a major role in the methoxamine-induced desensitization.

The relative insensitivity of endothelium-dependent effects to guanylate cyclase inhibitors in the rat mesenteric artery (Furchgott *et al*., 1987) raises the possibility that this effect may be mediated via other mechanisms. Recently we showed that the endothelium-dependent vasodilator response to ACh involves the release of EDHF, as well as NO, in the mesenteric arterial bed of the rat (Kamata *et al*., 1996). In the mesenteric arterial bed depolarized by exposure to an isotonic high K^+ medium, the second dose-response curve to methoxamine again showed a significant depression of the response by comparison with the first, indicating that EDHF is not involved to any extent in the methoxamin-induced desensitization. Pretreatment with capsaicin or tetrodotoxin did not affect the desensitization indicating that calcitonin gene-related peptide is probably not involved in the desensitization. There is much evidence that locally generated prostaglandins can play an important role in the desensitization that occurs with multiple applications of the adrenoceptor agonist, noradrenaline. However, preincubation with indomethacin did not affect the depression seen in the methoxamine-induced second dose-response curve in our mesenteric vascular bed preparations. This suggests that agonist-induced desensitization may not be related to the synthesis of prostanoids in the mesenteric arterial bed.

In conclusion, we found that the second dose-response curve for the methoxamine-induced increase in perfusion pressure in the mesenteric arterial bed showed a significant depression of the response by comparison with the first, and that this decrease in the response was significantly inhibited by the removal of the endothelium or by pretreatment with L-NOARG. The cyclic GMP level in the effluent from the perfused mesenteric arterial bed preparation was significantly increased after the second application of methoxamine, and this effect was also markedly inhibited by the removal of the endothelium or by pretreatment with L-NOARG. Our results suggest that a desensitization of the methoxamine-induced contraction develops rapidly in the mesenteric arterial bed, but not in the aorta, and that the release of NO from the endothelium plays a major role in this desensitization.

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