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Role of nitric oxide and nitric oxide-independent relaxing factor in contraction and relaxation of rabbit blood vessels

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Abstract

It has been shown that spontaneous release of nitric oxide (NO) from the vascular endothelium attenuates contractile responses of vascular smooth muscles to norepinephrine, and that acetylcholine-induced relaxation is mediated by the evoked release of NO and endothelium-derived hyperpolarizing factor. Since the involvement of these substances (or factors) in mechanical responses is heterogeneous among blood vessels, we have investigated the role of these substances in agonist-induced contraction and relaxation in 6 rabbit blood vessels. Vascular reactivity for the contractile response to norepinephrine was potentiated after removal of endothelium and by 100 μM N^G-nitro-L-arginine (L-NA) but not by 80 nM–0.4 μM clotrimazole. This potentiation was most marked in the mesenteric artery among the blood vessels tested, suggesting that the basal release of NO reduced the contractile response of the vascular smooth muscle to norepinephrine in this artery. Acetylcholine-induced relaxation was abolished by removal of the endothelium and was attenuated by L-NA (1–100 μM) in all blood vessels. The attenuation by 100 μM L-NA was most obvious in aorta and vein and least in mesenteric resistance artery in which the acetylcholine-induced, L-NA-resistant relaxation was inhibited by 80 nM–0.4 μM clotrimazole. These results suggested that there is a regional difference in the degree of involvement of NO in acetylcholine-induced relaxation. In mesenteric resistance artery, the NO-independent, clotrimazole-sensitive factor, possibly hyperpolarizing factor may also contribute to the response to acetylcholine at high concentrations. © 1997 Elsevier Science B.V.

Keywords: Acetylcholine; Clotrimazole; Endothelium; EDHF (endothelium-derived hyperpolarizing factor); Nitric oxide (NO); Nitro-L-arginine

1. Introduction

The vascular endothelium releases vasorelaxant substances, i.e., prostacyclin, endothelium-derived relaxing factor (EDRF), now identified as nitric oxide (NO) or closely related molecules and endothelium-derived hyperpolarizing factor (EDHF) (Chen et al., 1988; Lischke et al., 1995). These substances seem to act as local hormones to maintain vascular smooth muscle tone and to modify the reactivity of vascular smooth muscle to norepinephrine and acetylcholine.

Removal of the endothelium from blood vessels results in an increased sensitivity of contraction in response to α-adrenoceptor agonists in dog coronary and rabbit mesenteric arteries, rat and rabbit aortas and dog femoral vein (Malta et al., 1986; Martin et al., 1986; Miller, 1991; Moncada et al., 1991; Li and Kuriyama, 1993), but not in dog pulmonary vein and rat tail artery (De Mey and Vanhoutte, 1982; Tabernero et al., 1996). The responsiveness (maximum contractile response) to α_2-adrenoceptor agonists is enhanced after removal of the endothelium in dog coronary and rat pulmonary arteries and rat aorta (Bullock et al., 1986; Martin et al., 1986), whereas responsiveness to α_1-adrenoceptor agonists is facilitated (Carrier and White, 1985), decreased (De Mey and Vanhoutte, 1982) or unchanged in rabbit mesenteric, dog femoral and rat hepatic arteries and rat aorta (Malta et al., 1986; Bullock et al., 1986; Moncada et al., 1991; Zygmunt et al., 1994). The increase in the response has been suggested to result from enhancement of coupling between α_1-adrenoceptors and their cellular signalling pathway (Alosachie and Godfraind, 1988), a decrease in the amounts of agonist taken up by the endothelium (Carrier and White, 1985) and a lack of vasorelaxant action of NO, which is released either spontaneously (Moncada et al., 1991) or as a result of activation of α_2-adrenoceptors in the endothelium (Bullock et al., 1986; Miller, 1991). It has recently been

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demonstrated that NO synthase inhibitors increase the sensitivity of contraction in response to α_1- and α_2-adrenoceptor agonists in various types of vascular tissues (Mülsch and Busse, 1990; Miller, 1991; Moncada et al., 1991; Dowell et al., 1996). The responsiveness to partial agonists (2-[2-chloro-5-trifluormethyl-phenylimino]-imidazoline, St-587 and 5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline, UK 14304) is more markedly increased after endothelium denudation than is the responsiveness to full agonists (norepinephrine and phenylephrine) (Bullock et al., 1986; Martin et al., 1986; Tabernero et al., 1996). Thus, the underlying mechanisms of the endothelium-mediated modulation of contraction in response to α-adrenoceptor stimulants remain unclear.

Acetylcholine causes endothelium-dependent relaxation of vascular smooth muscles. The relaxation is mediated by the evoked release of NO and EDHF (Chen et al., 1988; Waldron and Garland, 1994; Lischke et al., 1995). EDHF seems to be cytochrome *P*-450-derived arachidonic acid metabolites, since cytochrome *P*-450 inhibitors such as clotrimazole and proadifen can attenuate the NO-independent relaxation (Hecker et al., 1994; Lischke et al., 1995; Zygmunt et al., 1996). In addition, EDHF is capable of producing hyperpolarization by opening potassium (K^+) channels in vascular smooth muscle cells (Harder et al., 1995). The role of EDHF in the acetylcholine-evoked relaxation seems to be more prominent in resistance than in large conduit arteries (Wu et al., 1993; Vicaut et al., 1994; Waldron and Garland, 1994).

Most studies have involved large arteries, although the relative contribution of NO and EDHF to the effects of norepinephrine and acetylcholine is tissue- and species-dependent. Therefore, we have studied whether or not the potentiation after endothelium removal of α-adrenoceptor agonist-elicited contractions can be explained by a reduced release of NO and EDHF in rabbit large and resistance arteries and vein. The arteries and vein chosen for the present experiments are known to contain α_1- and α_2-adrenoceptors, respectively (Muramatsu et al., 1990; Fujimoto and Itoh, 1995). Furthermore, the role of cytochrome *P*-450 products in acetylcholine-induced, N^G-nitro-L-arginine (L-NA)-resistant relaxation was investigated using the inhibitor of the *P*-450 pathway in the rabbit mesenteric resistance artery.

2. Materials and methods

2.1. Vascular preparations and tension measurement

Male Japan white rabbits (supplied from Kitayama Labs, Japan), weighing 1.9–2.3 kg, were anesthetized with pentobarbitone sodium (Nembutal, 40 mg/kg, i.v.) and exsanguinated by cutting through the left femoral artery. The abdominal aorta, common carotid, superior mesenteric and right femoral arteries and ear vein were excised. The second-order branches of the mesenteric artery were also used as resistance vessels. The blood vessels were cleaned of adventitial adipose and connective tissue and cut into 1.5- to 2-mm rings without side-branches in warmed (37°C) oxygenated Krebs-Henseleit bicarbonate (KHB) buffer of composition (mM): 114 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$ and 10 dextrose, pH 7.5. The rings were used for up to 2 days during which time no noticeable alterations in pharmacological responses were detected. In all cases, the KHB buffer contained 2 μM propranolol, 5 μM deoxycorticosterone, 0.2 μM desipramine and 10 μM indomethacin to avoid β-adrenoceptor-mediated relaxation, extraneuronal and neuronal uptakes of norepinephrine, and the possible production of vasoactive prostanoids, respectively (Fujimoto and Itoh, 1995). In some cases, the endothelium was removed from the preparation by gentle rubbing of the intimal surface with a metal wire before the ring was suspended. At the beginning of the experiment, successful removal of functional endothelium from the preparation was confirmed by the absence of relaxation in response to 0.3–1 μM acetylcholine in norepinephrine (1 μM)-contracted tissues.

The preparations were suspended under optimal resting tensions (aorta 2 g, carotid artery 1.5 g, mesenteric and femoral arteries 1 g, mesenteric resistance artery 300 mg and ear vein 150 mg) in 5 ml of the KHB buffer. The preparations were allowed to equilibrate for 60–90 min during which time the tension was re-adjusted if necessary, and then were contracted twice with 1–10 μM norepinephrine for 10 min at 30-min intervals. The tissues were washed by replacing the fresh KHB buffer every 10 min. Isometric tension changes were recorded through force-displacement transducers (TB-612T, Nihon Kohden Kogyo, Tokyo, Japan) coupled to a pen recorder.

2.2. Contractile responses to α-adrenoceptor agonists

In endothelium-intact and -denuded preparations, two cumulative concentration–response curves for α-adrenoceptor agonist-induced contractions were determined in the absence and presence of nitro-L-arginine (L-NA, 100 μM), clotrimazole (0.4 μM) or α-adrenoceptor antagonists, with an interval of 90 min between each determination. One of the paired preparations was treated for 60 min with these drugs before and during the determination of the second concentration–response curve; another untreated preparation was used to determine if any change in tissue sensitivity to the α-adrenoceptor agonists occurred in the course of the experiments (Fujimoto et al., 1988). Potencies of the α-adrenoceptor agonists were expressed as negative log EC_{50} values (pD_2 value), where the EC_{50} value was the molar concentration producing 50% of the maximum agonist response in the particular concentration–response curve.

2.3. Relaxation responses to acetylcholine and sodium nitroprusside

The blood vessels with endothelium were contracted with norepinephrine to 75–85% (EC_{75}) of the maximum norepinephrine response in the KHB buffer. After the contraction had reached steady state, a cumulative concentration–response curve for acetylcholine was made as follows: six sequential concentration–response curves for acetylcholine were made with an interval of 90 min between curves. One of the paired preparations was treated with increasing concentrations of L-NA (1–100 μM) 60 min before and during the determination of the second to the sixth concentration–response curves. Another untreated preparation was used as control. In some experiments, the concentration–response curves for acetylcholine in the presence of clotrimazole (80 nM–2 μM, 60 min) were made with an endothelium-intact mesenteric resistance artery in which the acetylcholine-induced, NO-dependent relaxation had been blocked by 100 μM L-NA. Cumulative concentration–response curves for sodium nitroprusside-induced relaxation were made with norepinephrine-stimulated blood vessels with and without endothelium. Relaxations were expressed as percentages of the decrease in the initial tone in response to norepinephrine.

The concentrations (EC_{75}) of norepinephrine used for contraction were as follows (in μM): endothelium-intact vs. -denuded: aorta 0.7 vs. 0.1, carotid artery 3 vs. 0.8, femoral artery 0.9 vs. 0.1, mesenteric artery 9 vs. 1, mesenteric resistance artery 2 vs. 0.9, and ear vein 0.1 vs. 0.05 (variation in means was less than 15%).

2.4. Drugs and solutions

The following drugs were dissolved in distilled water and diluted with the KHB buffer or 0.9% NaCl: acetylcholine chloride (Sigma, St. Louis, MO, USA), desipramine HCl (Sigma), N^G-nitro-L-arginine (Peptide Institute, Minoh, Japan), (−)-norepinephrine bitartrate (Sigma) and DL-propranolol HCl (Sigma). 5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK 14304, Research Biochemicals International, Natick, MA, USA), deoxycorticosterone acetate (Nakarai, Kyoto, Japan) and indomethacin (Sigma) were dissolved in ethanol. Clotrimazole (Sigma) was dissolved in dimethyl sulfoxide (Sigma). The final concentrations (less than 0.1%) of this solvent in the bathing medium had no noticeable effect on muscle contraction or relaxation.

2.5. Statistical analysis

The results are presented as mean values ± S.E. of the number (n) of observations. Statistical analysis was performed by using Student's t-test for non-paired data and differences were considered to be significant when $P < 0.05$.

3. Results

3.1. Contractile responses to α-adrenoceptor agonists

Norepinephrine (1 nM–0.1 mM) contracted rabbit blood vessel preparations in a concentration-dependent manner. The concentration–response curve for norepinephrine was shifted to the left after endothelium removal (Fig. 1). Sensitivity of the preparations to norepinephrine, as assessed by pD_2 values, is summarized in Table 1. Alterations in the sensitivity to norepinephrine after endothelium removal were nearly homogeneous (2.5–4 times) for all blood vessels studied, except mesenteric artery in which there was a 14-fold increase in sensitivity. Maximum norepinephrine responses in the endothelium-intact and -denuded tissues were as follows (mg, $n = 8–15$): aorta 2407 ± 169 and 2530 ± 145, carotid artery 1307 ± 35 and 1496 ± 100, femoral artery 1937 ± 124 and 2402 ± 142 ($P < 0.05$), mesenteric artery 1670 ± 53 and 1626 ± 264, mesenteric resistance artery 437 ± 32 and 488 ± 38, ear vein 146 ± 28 and 182 ± 27, respectively.

In endothelium-intact preparations, L-NA at 100 μM for 60 min did not significantly change the basal levels of vascular smooth muscle tone, and increased both the maxi-

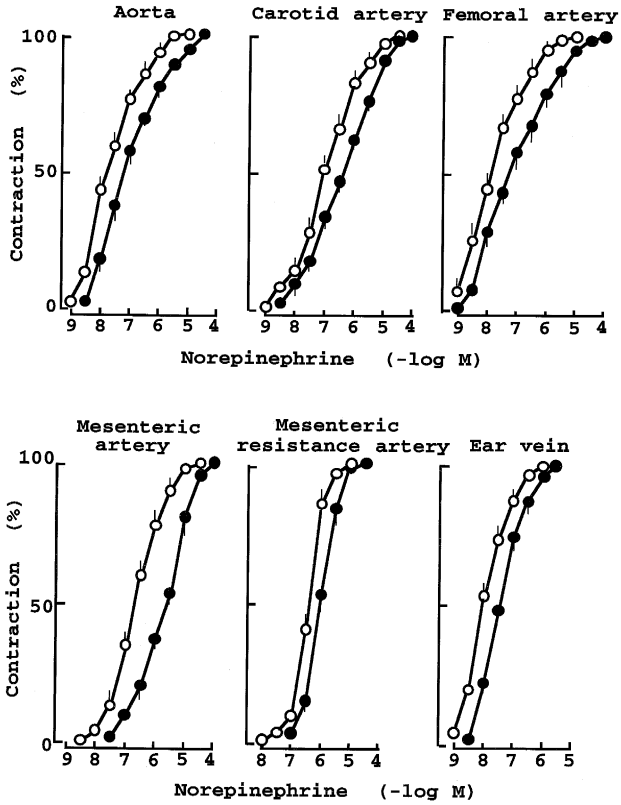

Fig. 1. Cumulative concentration–response curves for the norepinephrine-induced contraction in the rabbit aorta, carotid, femoral, mesenteric and mesenteric resistance arteries and ear vein with (●) and without (○) endothelium. Experiments were performed in the presence of 10 μM indomethacin. Ordinate: maximum norepinephrine responses are expressed as 100%. Vertical bars represent means ± S.E. ($n = 8–15$).

Table 1
Effects of nitro-L-arginine (L-NA) on norepinephrine-induced contraction in endothelium-intact and -denuded blood vessels of rabbit

	Aorta	Carotid artery	Femoral artery	Mesenteric artery	Mesenteric resistance artery	Ear vein
Endothelium-intact						
Control						
pD_2 value	7.11 ± 0.07	6.39 ± 0.04	7.32 ± 0.06	5.58 ± 0.08	6.05 ± 0.06	7.48 ± 0.06
L-NA (100 μM)						
Max. resp. (%) [a]	119 ± 3 [b]	115 ± 4 [b]	113 ± 6 [b]	139 ± 5 [b]	124 ± 4 [b]	146 ± 8 [b]
pD_2 value	7.41 ± 0.10 [b]	6.76 ± 0.08 [b]	7.64 ± 0.07 [b]	6.48 ± 0.11 [b]	6.37 ± 0.11 [b]	7.96 ± 0.07 [b]
Endothelium-denuded						
Control						
pD_2 value	7.73 ± 0.12 [c]	7.02 ± 0.10 [c]	7.85 ± 0.11 [c]	6.72 ± 0.08 [c]	6.42 ± 0.06 [c]	8.00 ± 0.06 [c]
L-NA (100 μm)						
Max. resp. (%) [a]	102 ± 3	99 ± 1	98 ± 3	101 ± 3	105 ± 2	93 ± 6
pD_2 value	7.78 ± 0.07	7.05 ± 0.08	7.95 ± 0.12	6.84 ± 0.13	6.37 ± 0.04	8.05 ± 0.04

Data are means ± S.E. ($n = 8–15$). [a] Maximum response is expressed as a percentage of the maximum norepinephrine response in the absence of L-NA. Significant difference with the corresponding control [b] and endothelium-intact tissues [c] ($P < 0.05$). The experiments were performed in the presence of 10 μM indomethacin.

mum norepinephrine response by 15–50% and, 2–3 times, the sensitivity in all vessels studied, again except mesenteric artery in which there was an 8-fold increase in sensitivity (Table 1). In endothelium-denuded rings, on the other hand, L-NA (100 μM) did not alter the responsiveness and sensitivity to norepinephrine. The pD_2 values for norepinephrine in the endothelium-denuded preparation were similar to those in the L-NA-treated endothelium-intact vessels.

Clotrimazole at 0.4 μM did not alter the sensitivity of any of the preparations to norepinephrine in the presence and absence of L-NA (Table 2). The drug did not alter the maximum norepinephrine response (data not shown).

UK 14304 contracted the carotid artery and ear vein at concentrations of 0.3–30 μM and 0.3 nM–1 μM, respectively, but did not cause significant contraction of the other types of arteries at concentrations lower than 10 μM. The amplitude of contraction induced by UK 14304 was 20%

Table 2
pD_2 values for norepinephrine-induced contraction of endothelium-intact blood vessels in the presence of clotrimazole and/or nitro-L-arginine

	Aorta	Carotid artery	Femoral artery	Mesenteric artery	Mesenteric resistance artery	Ear vein
Without L-NA						
Control	6.80 ± 0.03	6.09 ± 0.12	7.10 ± 0.06	6.00 ± 0.13	6.08 ± 0.09	7.66 ± 0.09
Clotrimazole (0.4 μM)	6.90 ± 0.03	6.12 ± 0.30	7.16 ± 0.10	6.10 ± 0.21	6.17 ± 0.14	7.82 ± 0.17
With L-NA (100 μM)						
Control	7.45 ± 0.10 [a]	6.90 ± 0.14 [a]	7.82 ± 0.11 [a]	6.40 ± 0.09 [a]	6.44 ± 0.04 [a]	8.09 ± 0.04 [a]
Clotrimazole (0.4 μM)	7.46 ± 0.15	6.85 ± 0.11	7.92 ± 0.15	6.48 ± 0.15	6.44 ± 0.06	8.06 ± 0.06

Results are given as means ± S.E. ($n = 3–8$). Significant difference from values obtained in the absence of L-NA ($P < 0.05$). The experiments were carried out in the presence of 10 μM indomethacin.

Table 3
Effects of nitro-L-arginine in UK 14304-induced contractions of carotid artery and ear vein

	Carotid artery		Ear vein	
	Endothelium (+)	Endothelium (−)	Endothelium (+)	Endothelium (−)
Control				
Max. res. (%) [a]	20.1 ± 2.1	34.1 ± 4.8 [b]	71.1 ± 4.2	118.5 ± 7.3 [b]
pD_2 value	5.17 ± 0.03	5.41 ± 0.05 [b]	8.03 ± 0.10	8.38 ± 0.10 [b]
L-NA (100 μM)				
Max. res. (%) [a]	49.4 ± 8.1 [c]	43.3 ± 6.2	149.4 ± 13.7 [c]	112.9 ± 8.5
pD_2 value	5.48 ± 0.06 [c]	5.20 ± 0.07	8.62 ± 0.16 [c]	8.46 ± 0.14

Results are expressed as means ± S.E. ($n = 4–12$). [a] Maximum response is expressed as a percentage of the maximum norepinephrine response in endothelium-intact tissue. Significant difference from endothelium-intact [b] and control vessel [c] ($P < 0.05$).

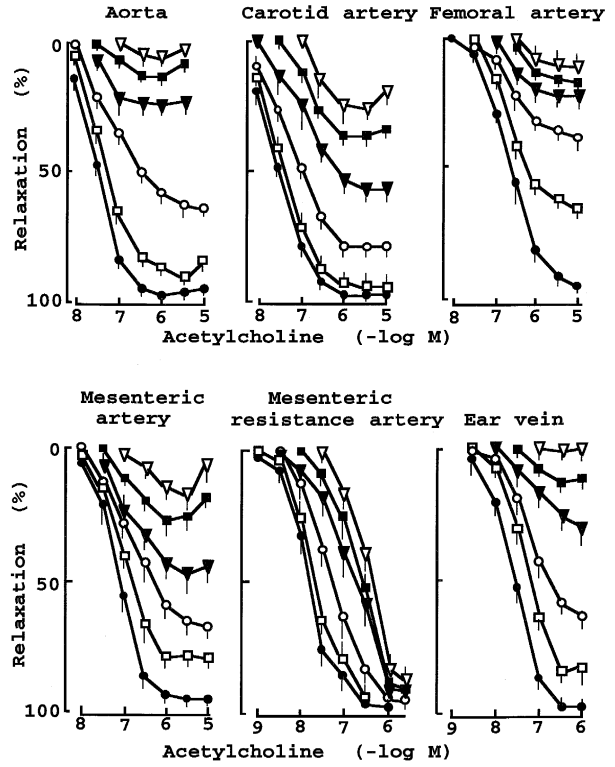

Fig. 2. Cumulative concentration–response curves for acetylcholine-induced relaxation in aorta, carotid, femoral, mesenteric and mesenteric resistance arteries and ear vein. The blood vessels were contracted with norepinephrine to 75–85% (ED_{75}) of the maximum agonist response either in the absence (●) or in the presence of L-NA at 1 (□), 3 (○), 10 (▼), 30 (■), and 100 (▽) μM. Relaxation was expressed as a percentage of the decrease in norepinephrine-induced contraction (ordinate). The norepinephrine contraction in the absence of L-NA was as follows (in mg): 1579±86 (aorta), 935±39 (carotid), 1716±52 (femoral), 1018±131 (mesenteric), 387±34 (mesenteric resistance) and 111±11 (vein). The experiment was carried out in the presence of 10 μM indomethacin. Vertical bars represent means±S.E. (n=6–8).

and 70% of the maximum norepinephrine responses in endothelium-intact carotid artery and ear vein, respectively (Table 3). Unlike that to norepinephrine, the maximum response to UK 14304 was increased 1.7 times by removal of the endothelium in both tissues. L-NA (100 μM) doubled the maximum response and enhanced the sensitivity to UK 14304 in the endothelium-intact artery and vein. L-NA was without effect in the endothelium-denuded preparations.

The ear vein was 700 times more sensitive to UK 14304 than was the carotid artery. Prazosin (5 nM) inhibited agonist effects of norepinephrine and UK 14304 in carotid artery but not in the ear vein, and the reverse was true with 30 nM yohimbine (data not shown).

3.2. Relaxation responses to acetylcholine and sodium nitroprusside

Acetylcholine (1 nM–10 μM) produced concentration-dependent relaxation of endothelium-intact preparations precontracted with norepinephrine in the presence of indomethacin (Fig. 2). L-NA reduced the ability of the tissues to relax in response to acetylcholine. Substantial differences in the degree of the inhibitory effect of L-NA existed among blood vessels tested: 1 μM L-NA inhibited the relaxation response in aorta, femoral, mesenteric arteries and ear vein, but not in carotid and mesenteric resistance arteries. In addition, 100 μM L-NA almost completely eliminated the response to acetylcholine in aorta and ear vein, but there remained a significant relaxation response in carotid, femoral and mesenteric arteries. In mesenteric resistance artery, the response to acetylcholine at concentrations less than 100 nM was completely abolished or well reduced by 30 μM L-NA, but the response to 1–3 μM acetylcholine was not as strongly suppressed by 100 μM L-NA as that in the other blood vessels.

Clotrimazole at a concentration of 2 μM altered the relaxation response of the mesenteric resistance artery to acetylcholine, but did not do so at 80 nM and 0.4 μM (Fig. 3). The response to acetylcholine in the presence of 100 μM L-NA was inhibited by clotrimazole at 80 nM–2 μM in a concentration-related manner. Clotrimazole at 2 μM slightly suppressed the maximum acetylcholine response.

Concentration–response curves for sodium nitroprusside are shown in Fig. 4. There were differences in sensitivity to sodium nitroprusside among the blood vessels. For instance, the EC_{50} values for sodium nitroprusside ranged between 0.01 μM (aorta and vein) and 1 μM (mesenteric

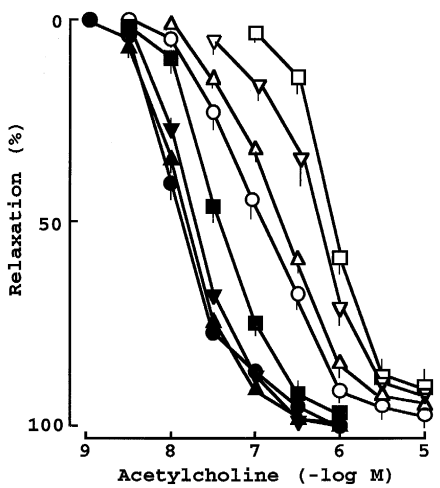

Fig. 3. Effects of clotrimazole on acetylcholine-induced relaxation of mesenteric resistance artery in the absence (closed symbols) and presence of L-NA (100 μM, open symbols). The tissues were exposed for 60 min to clotrimazole at concentrations of 80 nM (▲,△), 0.4 μM (▼,▽) and 2 μM (■,□). Control (●,○). Ordinate: contractile responses to norepinephrine at concentrations used (ED_{75}), expressed as 100%, were 354±28 mg (n=12) and 442±34 mg (n=9) in the absence and presence of L-NA, respectively. Clotrimazole at the concentrations used did not alter significantly either the basal level of the vascular tone or the ED_{75} value for norepinephrine. The experiments were performed in the presence of 10 μM indomethacin. Vertical bars indicate means±S.E.

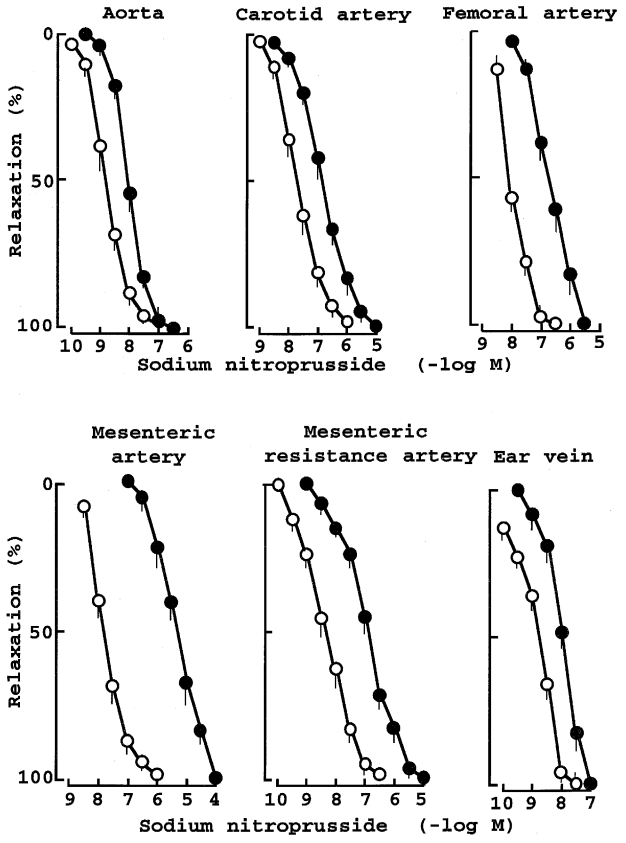

Fig. 4. Effects of sodium nitroprusside on norepinephrine-contraction of aorta, carotic, femoral, mesenteric and mesenteric resistance arteries and ear vein with (●) and without (○) endothelium. Ordinate: norepinephrine-induced contractions (expressed as 100%), in the endothelium-intact and -denuded vessels were as follows (in mg, $n = 4–7$): 1233 ± 165 vs. 1793 ± 67 (aorta), 788 ± 13 vs. 998 ± 92 (carotid), 1400 ± 50 vs. 1475 ± 238 (femoral), 838 ± 141 vs. 933 ± 93 (mesenteric), 392 ± 37 vs. 540 ± 75 (mesenteric resistance) and 171 ± 31 vs. 206 ± 29 (vein), respectively. Vertical bars represent means $\pm$ S.E. ($n = 4–7$).

artery), when the endothelium was intact. Endothelium removal enhanced the sensitivity without affecting the maximum response; the enhancement by endothelium removal was greatest in the mesenteric artery (300 times), least in aorta and ear vein (6 times) and intermediate in the other vessels (10–20 times). The regional differences in sensitivity decreased when the endothelium was removed.

4. Discussion

Several lines of evidence suggested that vascular endothelium releases NO spontaneously to decrease the reactivity of the underlying smooth muscle for the contractile response to α-adrenoceptor agonists (Bullock et al., 1986; Moncada et al., 1991; Zygmunt et al., 1994). The first aim of the present study was, therefore, to determine whether or not changes in regional variations in vascular reactivity to α-adrenoceptor agonists would follow removal of the endothelium, and furthermore whether or not endothelium-derived NO and EDHF are involved in these mechanical changes. It was found that the degree of supersensitivity of the response to α-adrenoceptor agonists after endothelial denudation was comparable in the large arteries (aorta, carotid and femoral), mesenteric resistance artery and ear vein, but was more marked in the mesenteric artery than in the other vessels. L-NA did not change the basal tone of the preparations (present experiment, Miller, 1991) and increased the sensitivity of the blood vessels to norepinephrine and the effect of L-NA disappeared completely in the endothelium-denuded vessels. Like that of de-endothelialization, the effect of L-NA was marked in the mesenteric artery. It is suggested that the supersensitivity after endothelium removal is in part the result of a decrease in the inhibitory effect of endothelium-derived NO. On the other hand, L-NA increased the maximum norepinephrine response in all endothelium-intact blood vessels studied. This result is consistent with previous observations (Mülsch and Busse, 1990; Moncada et al., 1991; Tabernero et al., 1996). On the contrary, endothelial denudation failed to increase the maximum responses to norepinephrine in dog and rat arteries and veins (Carrier and White, 1985; Bullock et al., 1986; Martin et al., 1986) and rabbit blood vessels except the femoral artery (present study). Thus, the lack of NO derived from the endothelium could not fully account for the changes in the vascular reactivity to norepinephrine that follow endothelial denudation. Alternatively, mechanical damage to the smooth muscle in the endothelium-denuded preparation may impair the increase in the maximum response.

Both the sensitivity and responsiveness to UK 14304 were increased by endothelium removal and by application of L-NA in endothelium-intact carotid artery and ear vein. The increase in the sensitivity to UK 14304 in either preparation was similar to that to norepinephrine. On the other hand, the increase in the responsiveness to UK 14304 was greater than that of the responsiveness to norepinephrine in both vein and artery. We found that UK 14304 acts as a full agonist at α_2-adrenoceptors in the vein and as a partial agonist at α_1-adrenoceptors in the artery, as suggested for rat aorta by Bullock et al. (1986). So, as compared to that with norepinephrine, the greater enhancement of UK 14304-induced contraction after endothelium removal was not related to the α_1- and α_2-adrenoceptor subtypes involved (artery vs. vein) nor with their relative efficacies on either receptor. However, it had been reported that the action of partial agonists was more effectively changed after removal of the endothelium than was that of full agonists (Tabernero et al., 1996) and that stimulation of the endothelial α_2-adrenoceptors evoked NO release (Bullock et al., 1986; Angus et al., 1986; Miller, 1991).

It was found that, of the blood vessels studied, aorta and vein, if the endothelium was present, were most sensitive to sodium nitroprusside (a NO donor) and mesenteric artery was least sensitive. When the endothelium was

removed, the sensitivity to sodium nitroprusside was increased. The result was consistent with previous data (Seidel and LaRochelle, 1987; Moncada et al., 1991; Dowell et al., 1996). The extent of the increase in sensitivity was heterogeneous, being greatest in mesenteric artery and least in aorta and ear vein. Inhibition of NO synthase also increased the sensitivity to sodium nitroprusside in aorta, mesenteric and mesenteric resistance arteries (Moncada et al., 1991; Li and Kuriyama, 1993; Dowell et al., 1996). These results suggest that, in the mesenteric artery, large amounts of NO are continuously released and this induces a consequent down-regulation of the receptor (the soluble guanylate cyclase) for NO or desensitization of the enzyme to the action of NO. Therefore, endothelial denudation was followed by an extremely pronounced supersensitivity. The present results, however, did not exclude the possibility that the mesenteric artery endothelium efficiently reduces the uptake of NO into smooth muscle cells.

Acetylcholine-induced relaxation of blood vessels consists of at least two components: one is inhibited by NO synthase inhibitors and the other is not. These components probably represent the evoked release of NO and EDHF, which has not yet been identified (Chen et al., 1988; Lischke et al., 1995). Recently, it was shown that cytochrome *P*-450 inhibitors, including clotrimazole, reduce NO synthase activity in bovine brain and aortic endothelium and inhibit NO-mediated relaxation in rat and rabbit aorta (Bennett et al., 1992; Wolff et al., 1993; Zembowicz et al., 1993; Oyekan et al., 1994; Li and Rand, 1996). Furthermore, it was suggested that *P*-450 inhibitors can inhibit EDHF formation and/or its effect on smooth muscle (Lischke et al., 1995; Edwards et al., 1996; Graier et al., 1996; Zygmunt et al., 1996). Since it is unclear to what extent EDHF contributes to the relaxation response to acetylcholine when NO production is or is not inhibited in blood vessels, we compared the effects of L-NA on acetylcholine-induced relaxation in the presence of indomethacin in large and resistant arteries and vein. It was found that L-NA inhibited acetylcholine-induced relaxation in all these vessels. The degree of the inhibition was heterogeneous: the inhibition by 100 μM L-NA was almost complete in aorta and ear vein, suggesting that, in these tissues, NO is a major substance mediating the response to acetylcholine in accordance with results of the study on rat aorta (Nagao et al., 1992; Zygmunt et al., 1994) and dog femoral vein (Miller, 1991). In the other arteries, there was a L-NA-resistant component in the response to acetylcholine. Particularly the maximum acetylcholine response of the mesenteric resistance artery was only slightly affected by 100 μM L-NA. Zygmunt et al. (1994) have shown that 300 μM L-NA does not suppress the acetylcholine-induced relaxation in rat hepatic artery. Therefore, acetylcholine-induced, NO-dependent and -independent relaxations were compared, using clotrimazole, in the mesenteric resistance artery. Clotrimazole at 2 μM inhibited the acetylcholine-induced relaxation of the artery in the absence of L-NA. In preliminary experiments, we found that 2 μM clotrimazole reduced both the acetylcholine-induced relaxation in aorta, and that in mesenteric resistance artery stimulated with 30 mM KCl and 0.8 μM norepinephrine. It has been reported that 100 μM clotrimazole did not inhibit relaxation response of vascular smooth muscle to sodium nitroprusside (Lischke et al., 1995). The drug could reduce NO production and/or release in these arteries. In the artery in which NO formation had been inhibited by L-NA, on the other hand, clotrimazole at the concentrations of 80 nM to 0.4 μM attenuated the acetylcholine-induced relaxation. Since the concentrations of clotrimazole used in the present study were 10–100-times lower than those necessary for inhibition of cytochrome *P*-450 (Edwards et al., 1996; Graier et al., 1996; Lischke et al., 1995; Zygmunt et al., 1996), cytochrome *P*-450-derived factors may not make the sole contribution to acetylcholine-induced, NO-independent relaxation and the underlying mechanisms of the clotrimazole-mediated inhibition on the relaxation to acetylcholine thus remain to be clarified.

In summary, contractile responses to norepinephrine and UK 14304 were potentiated by L-NA. Removal of the endothelium enhanced the sensitivity of the contractile response to these agonists and of the relaxant response to sodium nitroprusside, the enhancement being greatest in the mesenteric artery. In aorta and vein, the acetylcholine-induced relaxation was mainly mediated by NO. In contrast, there were both NO-dependent and to a lesser extent, L-NA-resistant relaxations in response to acetylcholine in the carotid, femoral and mesenteric arteries. In the mesenteric resistance artery, L-NA-resistant relaxation was clotrimazole-sensitive. These results indicate that the endothelium plays an important and variable role in the local regulation of vascular smooth muscle tone.

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