Br. J. Pharmacol. (1988), **95**, 531–543

Modulatory role of the vascular endothelium in the contractility of human isolated internal mammary artery

Philippe Schoeffter, *Robert Dion & [1]Théophile Godfraind

Laboratoire de Pharmacodynamie Générale et de Pharmacologie, Université Catholique de Louvain, Avenue Emmanuel Mounier 73, UCL 7350, B-1200 Bruxelles, Belgium and * Cliniques Universitaires Saint-Luc, avenue Hippocrate 10, B-1200 Bruxelles, Belgium

1 Endothelium-dependent relaxant responses and modulation of contractile responses were investigated in human isolated internal mammary artery (HIMA), a vessel widely used for coronary bypass surgery.

2 Acetylcholine and ionophore A23187 (both 10 nM–1 μM) elicited concentration-dependent relaxations of precontracted HIMA. These relaxations were abolished after rubbing of the endothelium, they were inhibited by methylene blue and were insensitive to indomethacin.

3 Histamine at concentrations lower than 10 μM elicited an endothelium-dependent, methylene blue-sensitive relaxation of precontracted HIMA. This effect of histamine was inhibited by the H_1-receptor antagonist mepyramine. Bradykinin, noradrenaline and α_2-adrenoceptor agonists (in the presence of prazosin) did not relax unrubbed HIMA in which acetylcholine or A23187 were shown to be efficient.

4 Tissue levels of guanosine-3':5'-monophosphate (cyclic GMP) were found to be significantly higher in unrubbed HIMA rings than in matched rubbed rings.

5 Methylene blue evoked a slow contraction in resting HIMA, and this contraction was significantly greater in unrubbed than in rubbed preparations. Also, methylene blue enhanced the contractile response of HIMA to noradrenaline and this potentiating effect was significantly greater in unrubbed than in rubbed preparations. Indomethacin induced a slow contraction, of similar magnitude in unrubbed and rubbed HIMA rings.

6 In resting HIMA, the concentration-effect curve of noradrenaline-induced contraction was significantly shifted to the left after rubbing of the endothelium, without change in the maximal responses. In unrubbed rings the EC_{50} value of noradrenaline was about 2 fold that in rubbed rings.

7 Histamine also contracted resting HIMA in a concentration-dependent manner and in addition, it triggered rhythmic activity. This rhythmic activity was more prominent in unrubbed preparations and could be partially inhibited by indomethacin. The concentration-effect curve of histamine-induced contractions was displaced to the left after rubbing the endothelium, without changes in the maximal responses. The EC_{50} value of histamine in unrubbed rings was 4 to 9 fold that found in rubbed rings, depending on the level of tension taken into account for the concentration-effect curve during rhythmic contractions.

8 In the presence of nifedipine (3 μM), noradrenaline-induced contractions were not significantly altered, whereas histamine-induced contractions were found to be inhibited by about 70%.

9 It is concluded that in HIMA, both spontaneous and stimulated endothelium-dependent relaxing factor (EDRF) release may occur, and that basal EDRF can itself be responsible for the modulatory effect of endothelium on contractile responses.

Introduction

The vascular endothelium plays an obligatory role in the vasorelaxation induced by a wide number of agents, as was first demonstrated by Furchgott & Zawadzki (1980) for acetylcholine. Since this discovery, relaxation of a great variety of isolated vessels by acetylcholine, histamine, bradykinin and other agents like the calcium ionophore A23187 has been shown to require the presence of an intact

[1] Author for correspondence.

endothelium (for reviews see Furchgott, 1984; Vanhoutte et al., 1986). There is in fact some evidence that these relaxing agents can trigger the liberation of endothelium-derived relaxing factor(s) (EDRF). Such a role for the vascular endothelium has also been reported in human isolated vessels, particularly human coronary arteries (Thom et al., 1985; Bossaller et al., 1986; Forstermann et al., 1986b).

In addition, the presence of endothelium can modulate agonist-induced contractile responses of some animal arteries (Cocks & Angus, 1983; Eglème et al., 1984; Miller et al., 1984; Lues & Schumann, 1984; Godfraind et al., 1985; Carrier & White, 1985; Miller & Vanhoutte, 1985), probably as a consequence of a spontaneous release of ('basal') EDRF (Malta et al., 1986a; Martin et al., 1986). To date, there has been no evidence for basal release of EDRF in human isolated arteries.

It was the purpose of the present work to examine the endothelium-mediated relaxant responses to various agents and the endothelium-mediated modulation of contractile responses in a human isolated artery. The human internal mammary artery (HIMA) was chosen because of its availability during aorto-coronary bypass surgery (Loop et al., 1986). Also, it was considered of importance to see whether endothelium-related events do actually occur in a vessel which is now widely used as a graft for coronary bypass.

The results show that, in this human artery, endothelial cells mediate the relaxation induced by some agents and that basal release of EDRF can modulate the contractile responses of the vessel to various agonists.

Methods

Biological material

HIMA from 35 patients (32 male, 3 female) were used in the present study. The mean age (+ s.e.mean) was 60 + 1 years. Fragments of right and left HIMA were obtained during coronary bypass surgical intervention. They were immediately immersed into cold Krebs bicarbonate solution of the following composition (in mM): NaCl 112, KCl 5, $CaCl_2$ 1.25, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1 and glucose 11.5. Tissues were then freed of adherent connective tissue and kept at 4°C in Krebs solution until use (that is maximally for 24 h). Three to four mm long rings were cut (external diameter 2–3 mm), care being taken not to damage the interior of the vessel. In some rings, the intimal surface was rubbed with a wooden matchstick or the ends of small forceps, in order to remove the endothelial cells. Such a procedure did remove these cells, as judged by histological examination of some of the preparations (according to Caplan & Schwartz, 1973) and the abolition of acetylcholine- or A23187-induced relaxation (see results).

Contractile experiments

Rubbed and unrubbed HIMA rings from the same vessel were mounted in parallel in 50 ml organ chambers containing Krebs solution aerated by a mixture of 95% O_2–5% CO_2 at 37°C. The preparations were suspended under a basal tension of 2 g and extensively washed during approximately 3 h, the tension being periodically readjusted to 2 g. After this period of time, the basal tension stabilized at about 1.5 g and it was not modified again. Isometric changes in tension were recorded. In all experiments, rings were first contracted by noradrenaline (1 μM) and after the contraction had fully developed either acetylcholine or the calcium ionophore A23187 was added to ensure that endothelium was present or not.

Tissues were then allowed to rest for 90 min. After this period of time, they were again contracted by noradrenaline (1–3 μM). When the contraction had reached a plateau, one or the other of the tested relaxing agents was added cumulatively, each concentration being allowed to develop its maximal effect. After another 90 min period of washing and resting, the same protocol was repeated after a 45 min-preincubation with indomethacin or a 20 min-preincubation with methylene blue, or (in the case of histamine used as a relaxing agent) after a 15 min-preincubation with mepyramine or cimetidine at the indicated concentrations. Each cycle of contraction-relaxation was separated from the preceding one by an interval of 90 min. Methylene blue was always added before the last contraction of a preparation, because of its partially irreversible effect. Relaxant responses were expressed as a percentage of the noradrenaline-induced increase in tension. When the effect of α-adrenoceptor agonists was investigated on precontracted HIMA, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 2 μM) was used to induce tone, in the presence of prazosin (1 μM) added 30 min beforehand.

In some HIMA preparations, after the first challenge with acetylcholine or A23187, cumulative concentration-effect curves of histamine- or noradrenaline-induced contractions were performed in non-precontracted (resting) arteries, and repeated 90 min later in the presence or the absence of nifedipine or indomethacin.

The K_B value of mepyramine has been calculated according to Furchgott (1972).

Measurements of tissue levels of guanosine 3':5' monophosphate (cyclic GMP)

Matched rubbed and unrubbed HIMA rings prepared as for contractile experiments were incubated without tension in the Krebs solution described above, bubbled with 95% O_2:5% CO_2, at 37°C, during 3 h and the bathing solution was changed periodically. After this, tissues were quickly taken out of the bath and frozen using aluminium clamps precooled in liquid N_2. They were then thawed in 1 N $HClO_4$ and homogenized in a glass/glass Potter apparatus. Cyclic GMP was assayed by the radioimmunological method of Cailla *et al.* (1976), including a succinylation step. The iodinated derivative of cyclic GMP, namely [^{125}I]-succinyl-cyclic GMP-tyrosylmethylester was prepared by V. Schini (Laboratoire de Pharmacodynamie, Université Louis Pasteur, Strasbourg, France) and the anti-cyclic GMP specific antibody was supplied by Immunotech (Marseille-Luminy, France). Cyclic GMP levels were expressed with reference to the protein content, as determined according to Lowry *et al.* (1951), using bovine serum albumin as a standard.

Drugs

Noradrenaline (Fluka) was prepared as a stock solution (10 mM) in 7.9 mM Na_2SO_3 and 34 mM HCl and kept at 4°C. Acetylcholine chloride (Merck) was dissolved in 0.1 M NaH_2PO_4 to provide a stock solution (10 mM) kept at −20°C until use. Ionophore A23187 (Boehringer-Mannheim), prazosin hydrochloride (a gift from Pfizer) and nifedipine (a gift from Bayer) were prepared as stock solutions of respectively 1 mM, 1 mM and 10 mM in absolute ethanol and diluted with distilled water to the appropriate concentrations. Prostaglandin $F_{2\alpha}$ was obtained from Upjohn as Dinolytic. Indomethacin (Sigma) was prepared at 10 mM in 50 mM Na_2CO_3.

Histamine dihydrochloride (Merck, Darmstadt), bradykinin (UCB, Brussels), clonidine hydrochloride (a gift from Boehringer-Ingelheim), UK 14,304 tartrate (a gift from Pfizer), cimetidine (a gift from Smith, Kline and French), mepyramine hydrochloride (a gift from Specia) and methylene blue (Merck) were dissolved in distilled water.

Statistical analysis of the data

Results are expressed as means ± s.e.mean, except EC_{50} values which are given as geometric means with 95% confidence limits indicated in brackets. The Mann-Whitney U test and the Wilcoxon test were used for statistical comparisons of unpaired and paired values, respectively. P values less than 0.05 were considered significant.

Results

Endothelium-dependent relaxation of HIMA

Acetylcholine (10 nM–10 μM) relaxed unrubbed HIMA precontracted by noradrenaline (1 μM) in a concentration-dependent manner, but did not elicit relaxation of matched rubbed preparations (Figure 1a). The EC_{50} value of acetylcholine in unrubbed HIMA was 79 nM (15–209 nM, $n = 6$) and a maximal effect of about 65% relaxation was reached with 1 μM acetylcholine (Figure 2a). This effect was not significantly altered after pretreatment with indomethacin (10 μM), but significantly depressed after pretreatment with methylene blue (3 μM; Figure 2a). In the latter case, acetylcholine relaxed noradrenaline-precontracted HIMA by only 9.2 ± 6.2% ($n = 4$), and the EC_{50} value could not be accurately measured.

The calcium ionophore A23187 (10 nM–1 μM) also elicited a concentration-dependent relaxation of unrubbed, but not of rubbed HIMA precontracted by noradrenaline (Figure 1b). A maximal relaxation of about 80% and an EC_{50} value of 67 nM (29–158 nM, $n = 6$) were found (Figure 2b). Pretreatment of unrubbed HIMA with indomethacin (10 μM) did not significantly alter the relaxing effect of compound A23187. Methylene blue inhibited A23187-elicited relaxation less efficiently than acetylcholine-elicited relaxation. Nevertheless this inhibition was concentration-dependent. At a concentration of 3 μM, the dye significantly reduced the relaxation induced by 0.1 μM A23187, without significant change in the maximal effect of the ionophore (Figure 2b). A tenfold higher concentration of methylene blue (30 μM) significantly reduced relaxant responses elicited by 0.1 to 1 μM A23187. The EC_{50} values of A23187 in the presence of 3 and 30 μM methylene blue were 158 nM (87–288 nM, $n = 7$) and 161 nM (73–354 nM, $n = 5$), respectively. Both these values were significantly ($P < 0.05$) greater than that found in the absence of methylene blue.

Histamine (0.3 μM–3 μM) was able to relax unrubbed HIMA precontracted by noradrenaline, in a concentration-dependent manner (Figures 1c and 3), whereas it evoked further contraction of matched rubbed HIMA. At concentrations higher than 3 μM, the relaxant effect of histamine in unrubbed preparations was reversed and contraction predominated (Figure 1c). Pretreatment by methylene blue (3 μM) abolished the relaxant effect of histamine in unrubbed arteries (Figure 3). The relaxation induced

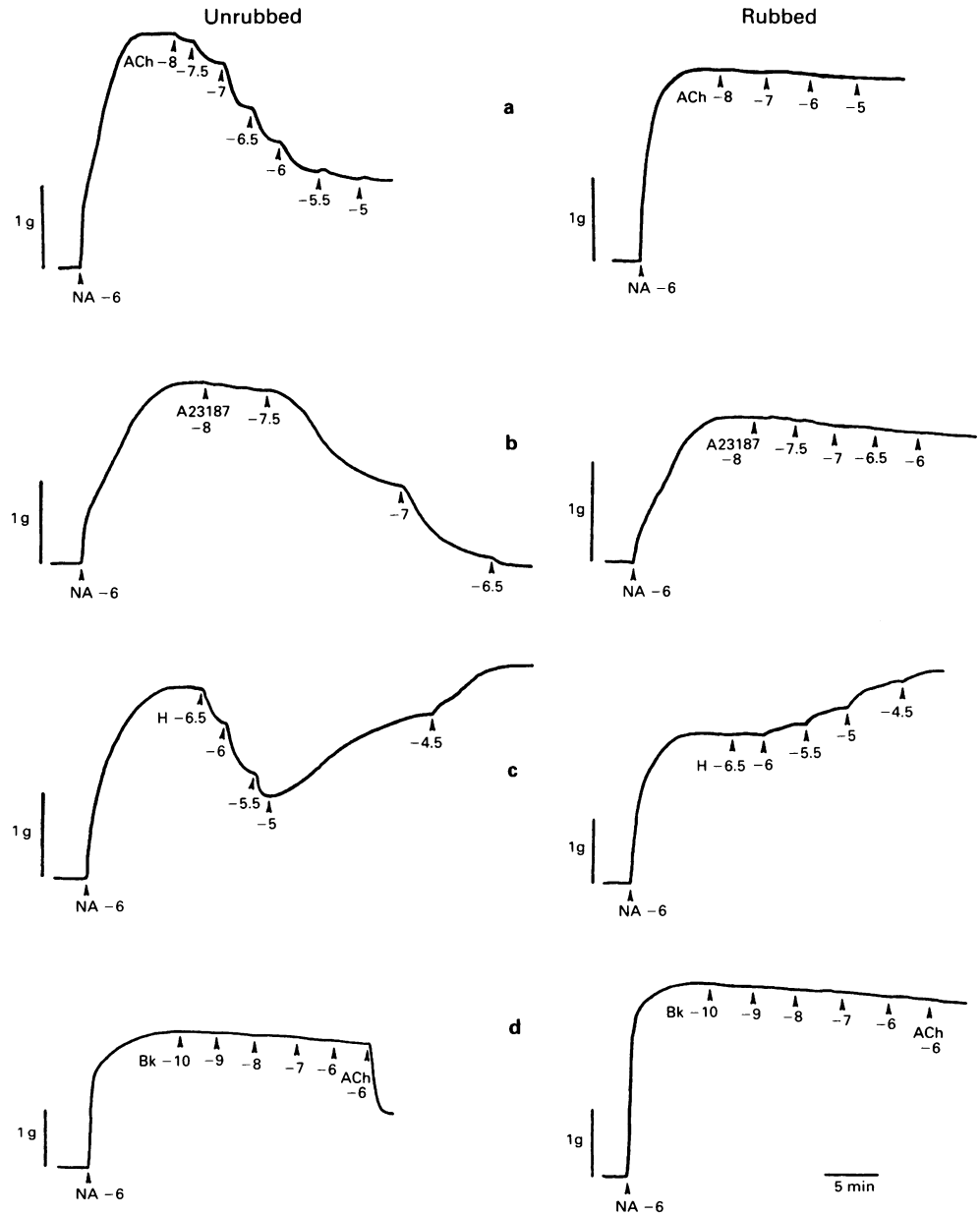

Figure 1 Typical recordings of the effect of (a) acetylcholine (ACh), (b) compound A23187, (c) histamine (H) and (d) bradykinin (Bk) on unrubbed and rubbed rings of human internal mammary artery (HIMA) precontracted by noradrenaline (NA). Concentrations are expressed in log units.

by histamine in unrubbed HIMA was antagonized by mepyramine (1 μM) in an apparently competitive manner, but not significantly affected by cimetidine (10 μM; Figure 4). From the mean curves represented in Figure 4, a pK_B ($-\log K_B$) value of 7.8 was calculated for mepyramine.

Bradykinin (0.1 nM–1 μM) was devoid of effect on noradrenaline-precontracted HIMA, either rubbed

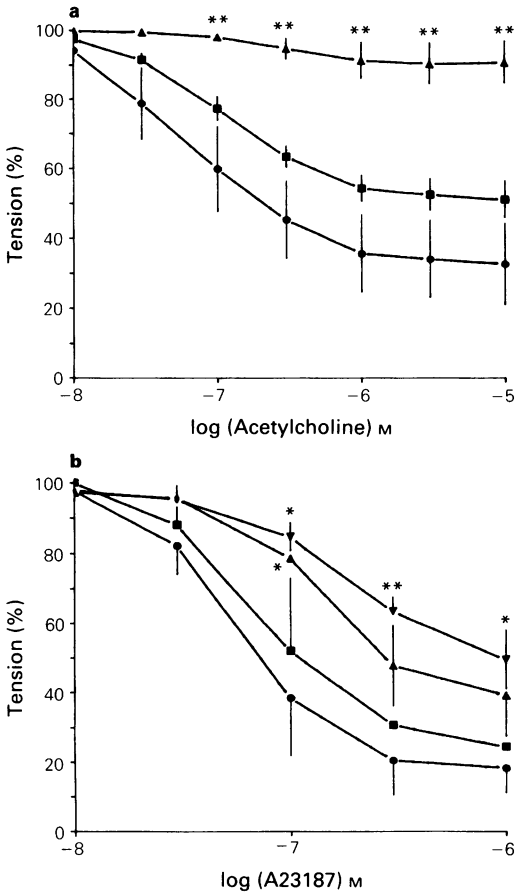

Figure 2 Concentration-effect curves of (a) acetylcholine- and (b) A23187-induced relaxation of unrubbed precontracted HIMA, in (●) the absence and in the presence of (■) indomethacin (10 μM) or methylene blue [3 μM (▲) and 30 μM (▼)]. (a) Results are the means ± s.e.mean (indicated by vertical bars) of 6 (●), 3 (■) and 4 (▲) observations. Noradrenaline-induced precontractions were 2.20 ± 0.58 g (●), 3.23 ± 1.24 g (■) and 3.28 ± 0.94 g (▲). (b) Results are the means ± s.e.mean (indicated by vertical bars) of 6 (●), 3 (■), 7 (▲) and 5 (▼) observations. Noradrenaline-induced precontractions were 2.71 ± 0.76 g (●), 3.50 ± 0.59 g (■), 2.55 ± 0.88 g (▲) and 1.11 ± 0.13 g (▼). Statistical significance is shown with respect to corresponding values in the absence of pretreatment: $*P < 0.05$; $**P < 0.01$.

or unrubbed (Figure 1d). This observation was reproduced in 5 different arteries, in which acetylcholine or A23187 elicited an endothelium-dependent relaxation.

Stimulatoin of endothelial α_2-adrenoceptors has been shown to elicit relaxation in some types of

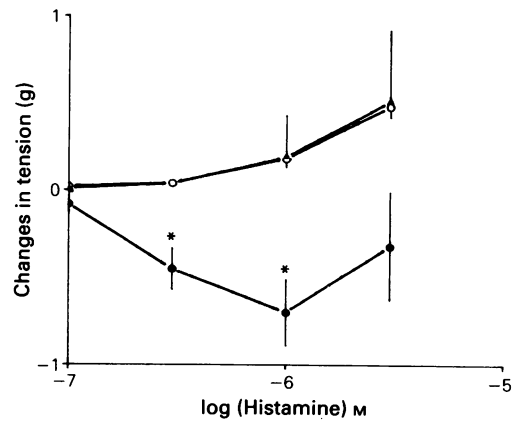

Figure 3 Changes in tension induced by histamine (0.1–3 μM) in precontracted (○) rubbed and unrubbed HIMA in (●) the absence and (▲) the presence of methylene blue (3 μM). Results are the means ± s.e.mean (indicated by vertical bars) of 5 (○), 5 (●) and 3 (▲) observations. Noradrenaline-induced precontractions were 1.98 ± 0.60 g (○), 1.72 ± 0.44 g (●) and 2.72 ± 0.99 g (▲). Statistical significance is shown with respect to corresponding values in rubbed preparations or in unrubbed preparations pretreated by methylene blue: $*P < 0.05$.

animal arteries (Cocks & Angus, 1983; Miller & Vanhoutte, 1985; Angus *et al.*, 1986a,b; Bullock *et al.*, 1986). In unrubbed HIMA precontracted by $PGF_{2\alpha}$ in the presence of 1 μM prazosin, the

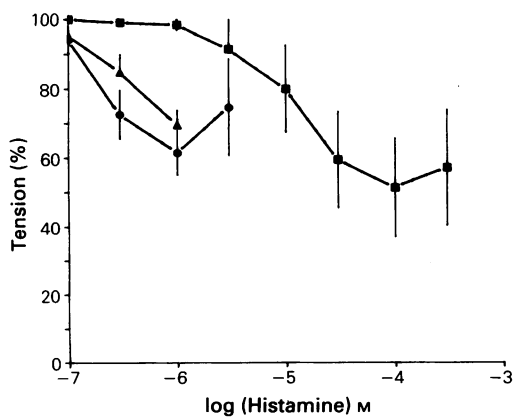

Figure 4 Relaxation of unrubbed precontracted HIMA in (●) the absence and in the presence of (▲) cimetidine (10 μM) or (■) (mepyramine (1 μM). Results are the means ± s.e.mean (represented by vertical bars) of 5 (●), 3 (▲) and 3 (■) observations. Noradrenaline-induced precontractions were 1.98 ± 0.60 g (●), 2.40 ± 1.02 g (▲) and 2.01 ± 0.64 g (■).

α_2-adrenoceptor agonists clonidine (10 nM–10 μM) and UK 14,304 (0.1 μM–10 μM) were devoid of any relaxing activity ($n = 3$ and 2, respectively), although the preparations could be relaxed by acetylcholine.

Evidence for a spontaneous release of (basal) EDRF in HIMA

Removal of the endothelium in various arteries isolated from animal species lowers the tissue levels of guanosine 3':5'-monophosphate (cyclic GMP; Holzmann, 1982; Rapoport & Murad, 1983; Diamond & Chu, 1983). As a consequence, the tissue levels of cyclic GMP have been considered as an index of spontaneous release of basal EDRF. Upon removal of the endothelium from HIMA rings, cyclic GMP levels were significantly ($P < 0.05$) decreased from 2.4 ± 0.2 pmol mg^{-1} protein in unrubbed rings to 1.7 ± 0.2 pmol mg^{-1} protein in matched rubbed rings ($n = 6$), that is a mean fall of 30%.

Another indication of spontaneous EDRF release can be given by the greater contracting effect of the guanylate cyclase inhibitor, methylene blue and the greater ability of this compound to potentiate agonist-induced contractions of unrubbed as compared to rubbed vascular tissues (Griffith *et al.*, 1985; Martin *et al.*, 1985; Murakami *et al.*, 1985). In HIMA rings, methylene blue (3 μM) elicited a slowly developing contraction which was significantly greater in unrubbed than in matched rubbed preparations (Table 1). Furthermore, in the presence of methylene blue (3 μM) the contraction elicited by noradrenaline (1 μM) was enhanced to a significantly greater extent in unrubbed than in rubbed arteries (Table 1).

The cyclo-oxygenase inhibitor indomethacin (10 μM) was also able to elicit a slow contraction of HIMA rings, which was quite similar in unrubbed and rubbed preparations (Table 1). In addition, pretreatment by indomethacin (10 μM) had not a significantly more marked effect on noradrenaline-induced contraction of unrubbed than in that of rubbed preparations (Table 1). Since the contracting effect of indomethacin in isolated vessels is attributable to the suppression of vasodilator prostanoids (Roberts *et al.*, 1981; Rubanyi & Vanhoutte, 1985), the latter results indicate that the contribution of these prostanoids to the modulation of the basal tone is not greater in unrubbed HIMA than it is in rubbed HIMA.

Endothelium-dependent modulation of HIMA contractions

Noradrenaline elicited a concentration-dependent contraction of resting unrubbed HIMA within the range of 30 nM–10 μM (Figure 5), with an EC_{50} of

Table 1 Contracting effect and potentiation of the effect of noradrenaline induced by methylene blue and indomethacin in rubbed and unrubbed human isolated internal mammary artery (HIMA)

	Rubbed	*Unrubbed*	n
Methylene blue (3 μM)			
Contracting effect	0.10 ± 0.03 g	0.26 ± 0.05 g*	11
Potentiation of the effect of noradrenaline	128 ± 10%	194 ± 39%*	10
Indomethacin (10 μM)			
Contracting effect	0.50 ± 0.11 g	0.44 ± 0.06 g	7
Potentiation of the effect of noradrenaline	104 ± 4%	109 ± 4%	7

Results are the means ± s.e.mean of n observations. The potentiation of noradrenaline-induced contraction has been quantified as the amplitude of noradrenaline (1 μM)-induced contraction in the presence of indomethacin or methylene blue expressed as a percentage of the amplitude of contraction in the absence of indomethacin or methylene blue. Statistical significance is given between corresponding values in rubbed and unrubbed HIMA: *$P < 0.05$. Indomethacin and methylene blue were left in contact for 45 and 20 min, respectively, before the addition of noradrenaline.

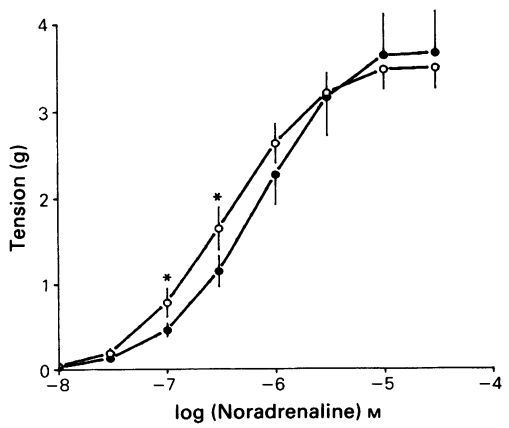

Figure 5 Concentration-effect curves of noradrenaline-induced contraction in (●) unrubbed and (○) rubbed resting HIMA rings. Results are the means of 11 observations (from 6 different patients) ± s.e.mean (represented by vertical bars). Statistical significance is given between corresponding values in unrubbed and rubbed tissues: *$P < 0.05$.

0.65 μM (0.52–0.79 μM, $n = 11$). Upon removal of the endothelium, the concentration-effect curve for noradrenaline-induced contraction was slightly but significantly shifted to the left without significant alteration in the maximal effect. The EC_{50} value of noradrenaline in rubbed HIMA was significantly ($P < 0.01$) reduced to 0.31 μM (0.19–0.51 μM, $n = 11$), that is about two fold less than that found in matched unrubbed HIMA.

In resting unrubbed HIMA, histamine (1 μM–1 mM) induced a concentration-dependent contraction, with marked rhythmic activity appearing over the middle range of histamine concentrations (3 μM–0.1 mM; Figure 6a). This rhythmic activity could be attenuated after pretreatment by indomethacin and it was also greatly reduced or sometimes abolished in rubbed HIMA arteries (Figure 6b). Moreover, rubbed HIMA preparations were more sensitive to histamine than were matched unrubbed preparations, as shown by the concentration-effect curves for histamine (Figure 7). Figure 7 represents the concentration-effect curves for histamine-induced contraction in matched unrubbed and rubbed HIMA, when measuring either the maximal or the minimal tension developed during rhythmic oscillations of tension after each addition of histamine. Thus, when considering the lower levels of tension developed during rhythmic oscillations, the EC_{50} value for histamine was significantly ($P < 0.01$) decreased after removal of the endothelium, from 28.2 μM (12.3–64.6 μM, $n = 12$) to 3.2 μM (2.4–4.2 μM, $n = 12$), that is about 9 fold less in rubbed rings than in unrubbed rings. When the higher levels of oscillatory tension were taken into consideration, the EC_{50} value for histamine was significantly ($P < 0.01$) decreased from 8.9 μM (3.1–25.7 μM, $n = 12$) to 2.3 μM (1.7–3.0 μM, $n = 12$) after removal of the endothelium, that is about 4 fold less in rubbed ring than in unrubbed rings. The maximal contracting effect of histamine was not significantly different in rubbed as compared to that in unrubbed HIMA (Figure 7).

It has been shown that agonist-induced contractions of vascular smooth muscle which are relatively resistant to the modulatory effect of the endothelium are also relatively resistant to the effect of calcium entry blockers (Eglème *et al.*, 1984; Godfraind *et al.*, 1985). Therefore it has been proposed that EDRF could affect receptor-operated Ca^{2+}-gating in vascular smooth muscle (Godfraind, 1986). We have investigated the effect of nifedipine, a dihydropyridine compound which blocks Ca^{2+} entry into vascular smooth muscle cells (Godfraind 1982), on noradrenaline- and histamine-elicited contractions of HIMA. Pretreatment with nifedipine (3 μM, 90 min) did not significantly alter noradrenaline-induced contractions of unrubbed HIMA (Figure 8a). By contrast, nifedipine depressed the contraction

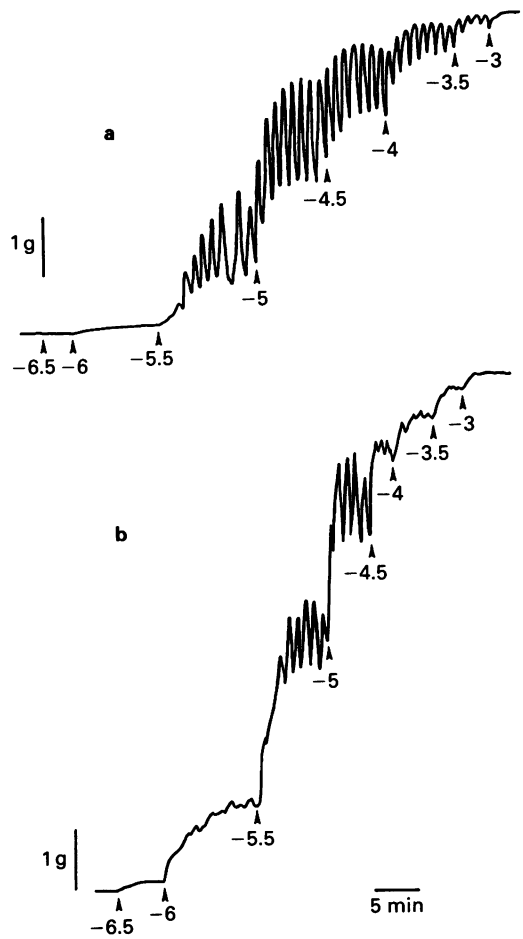

Figure 6 Typical recording of the contraction induced by cumulative addition of increasing concentrations of histamine (3 μM–1 mM) to (a) an unrubbed and (b) a rubbed resting HIMA preparation. Concentrations are expressed in log units. Note the rhythmic activity triggered by histamine (3 μM–0.3 mM) particularly in the unrubbed preparation.

induced by histamine in unrubbed HIMA and abolished the rhythmic activity triggered by histamine. Thus, as compared to the lower level of tension developed during rhythmic contractions induced by histamine in control unrubbed HIMA (see Figure 7), histamine-induced contractions were depressed by about 70% in the presence of nifedipine (Figure 8b).

Discussion

In the present study, we provide some evidence that the presence of the endothelium in human internal mammary artery (1) is obligatory for the relaxing

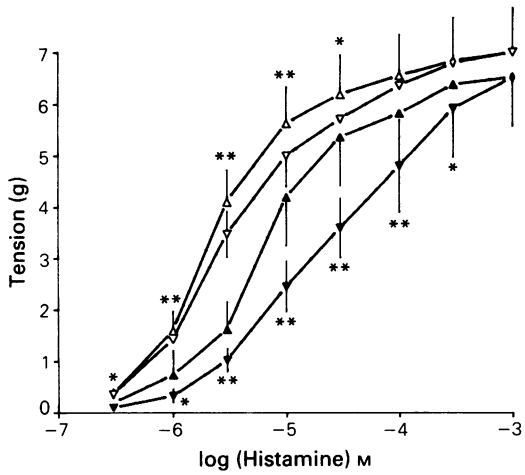

Figure 7 Concentration-effect curves of histamine-induced contraction in unrubbed (closed symbols) and rubbed (open symbols) resting HIMA. Also represented are the higher (triangles) and lower (inverted triangles) levels of contraction developed during rhythmic oscillations (see Figure 6). Results are the mean of 12 observations (from 8 different patients) ± s.e.mean (represented by vertical bars). Statistical significance is given between corresponding values in unrubbed and rubbed tissues, either considering the higher or the lower tension developed during rhythmic contractions: $*P < 0.05$; $**P < 0.01$.

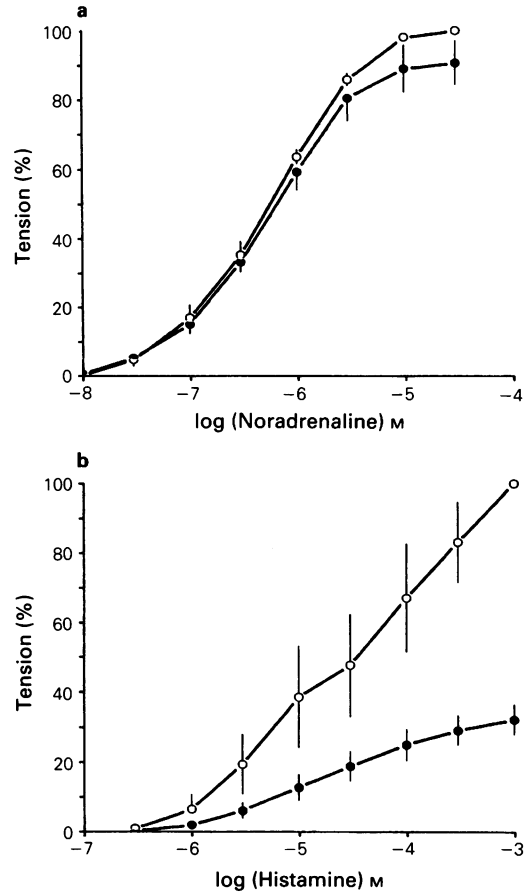

Figure 8 Concentration-effect curves for (a) noradrenaline- and (b) histamine-induced contraction in unrubbed resting HIMA, in (○) the absence and in (●) the presence of nifedipine (3 μM, added 90 min before the first addition of contracting agent). Results are the means ± s.e.mean (represented by vertical bars) of 4 observations. Contractions are expressed as a percentage of the maximal tension developed by control preparations (in the absence of nifedipine). Note that in the case of histamine (b), the lower level of tension developed during rhythmic oscillations has been taken into consideration for the control curve.

action of some agents and (2) can modulate agonist-induced contraction. These results are particularly interesting in view of the growing use of HIMA as graft for coronary artery bypass (Loop *et al.*, 1986). So far as endothelial-related vascular responses are concerned, these results make HIMA a good substitute for human epicardial coronary artery, whose relaxation has been reported to involve endothelial cells in some instances (Ginsburg & Zera, 1984; Thom *et al.*, 1985; 1986; Bossaller *et al.*, 1986; Forstermann *et al.*, 1986b).

Endothelial mediated acetylcholine- and A23187-elicited relaxations of HIMA exhibit the typical features of endothelium-dependent relaxation of animal arteries (e.g. rabbit aorta) by these agents. In rabbit aorta acetylcholine- and A23187-induced relaxations have been ascribed to the stimulated release of an endothelium-derived relaxing factor or EDRF (Furchgott & Zawadzki, 1980; Furchgott, 1983). The active concentration range of acetylcholine and A23187 was 10 nM–1 μM; their relaxing effect was not significantly altered after pretreatment with indomethacin, making it unlikely that any prostanoid is EDRF; on the contrary, the guanylate cyclase inhibitor methylene blue significantly inhibited acetylcholine- and A23187-induced relaxation of HIMA, indicating that cyclic GMP is involved in these relaxations, as was first suggested for bovine coronary arteries (Holzmann, 1982) and in the aorta of the rat (Rapoport & Murad, 1983) and of the rabbit (Diamond & Chu, 1983).

The differential sensitivity of acetylcholine- and A23187-elicited relaxations of HIMA to inhibition by methylene blue suggests that the two relaxing agents may act through distinct EDRFs. Although strong evidence for this contention is lacking, it is noteworthy that A23187-elicited relaxation, unlike

cholinergic relaxation, is not inhibited by quinacrine in rat and rabbit aorta (Zawadzki *et al.*, 1980; Furchgott, 1981; Rapoport & Murad, 1983; Singer & Peach, 1983). Furthermore, A23187-elicited relaxation is less sensitive to inhibition by nordihydroguaiaretic acid than is cholinergic relaxation in rabbit aorta (Singer & Peach, 1983). An alternative explanation would be that methylene blue, in addition to being an inhibitor of guanylate cyclase, could interact with a receptor-mediated process leading to EDRF release.

Histamine could elicit an endothelium-dependent relaxation of precontracted HIMA, but this effect was limited to lower concentrations (0.1–1 μM). At concentrations higher than 1 μM, the relaxant effect of histamine in unrubbed HIMA was reversed and the contracting effect predominated. In rat isolated aorta, histamine, like acetylcholine has been proved to stimulate the release of EDRF (Van de Voorde & Leusen, 1983) and histamine-induced endothelium-dependent relaxation has been associated with an increase in tissue levels of cyclic GMP (Rapoport & Murad, 1983). That methylene blue abolished the relaxant effect of histamine in unrubbed HIMA supports the involvement of cyclic GMP in this relaxation. On the other hand, histamine-induced relaxation of HIMA was antagonized by mepyramine but not by cimetidine. This indicates that endothelial histamine receptors are of the H_1 subtype in HIMA, an observation similar to those made in rat aorta (Van de Voorde & Leusen, 1983; Davies & Williams, 1984; Carrier *et al.*, 1984), guinea-pig pulmonary artery (Satoh & Inui, 1984), monkey coronary arteries (Toda, 1986), rat mesenteric bed (Byfield & Swane, 1987) and human umbilical vessels (Leusen & Van de Voorde, 1986). The calculated pK_B value of mepyramine (7.8) is relatively lower than the classical pA_2 value of the H_1-receptor antagonist (about 9, see Ash & Schild, 1966). This difference could be explained by effects of histamine on other receptor sites (H_1 and H_2) on the smooth muscle of HIMA. A similar difference between classical and calculated K_B value has been observed for another H_1-receptor antagonist, diphenhydramine, in the case of histamine-elicited endothelium-dependent relaxation of rat aorta (Carrier *et al.*, 1984). Thus, in HIMA there is some evidence that histamine can induce the release of EDRF via stimulation of an endothelial H_1 receptor.

Bradykinin is able to elicit an endothelium-dependent relaxation of a large variety of canine arteries (Chand & Altura, 1981; Cherry *et al.*, 1982). Such a bradykinin-induced relaxation was also found to occur in pig aorta (Gordon & Martin, 1983), in bovine intrapulmonary artery and vein (Gruetter & Lemke, 1986a) and in bovine coronary artery (Angus *et al.*, 1986b). By contrast, the aorta and the renal arteries of neither the cat (Furchgott, 1983) nor the rabbit (Cherry *et al.*, 1982) were relaxed by bradykinin, and in mesenteric arteries of the same species the relaxing effect of bradykinin was proved to be endothelium-independent (Cherry *et al.*, 1982; Forstermann *et al.*, 1986a). So far as human vessels are concerned, Furchgott and coworkers have reported an endothelium-dependent relaxation elicited by bradykinin in human mesenteric arteries (Cherry *et al.*, 1982) and ovarian artery (Furchgott, 1983). A similar observation was made in human basilar artery (Whalley *et al.*, 1987). On the other hand, Thom *et al.* (1986) did not observe any relaxant effect of bradykinin in human coronary arteries whose endothelium was otherwise shown to be functional. In HIMA we found no evidence of a relaxation induced by bradykinin, either in unrubbed or rubbed preparations. It appears therefore that bradykinin-induced endothelium-dependent relaxation is not a general observation either in human vascular beds or in animal ones.

In some precontracted animal vessels, noradrenaline and α_2-adrenoceptor agonists could elicit an endothelium-dependent relaxation, in particular after blockade of the α_1-adrenoceptors which mediate the contracting effect of noradrenaline (Cocks & Angus, 1983; Miller & Vanhoutte, 1985; Angus *et al.*, 1986a,b; Bullock *et al.*, 1986). However, this is not a general observation, since it could not be demonstrated in all types of arteries from the same species (Angus *et al.*, 1986a,b). Such a relaxing effect of noradrenaline has also been observed in human pulmonary artery, although reportedly not in a reproducible manner (Thom *et al.*, 1985). In unrubbed HIMA we could not demonstrate any relaxing effect of noradrenaline or of the α_2-adrenoceptor agonists clonidine and UK 14,304, after blockade of the α_1-adrenoceptors. These results do not support the existence of endothelial α_2-adrenoceptors mediating relaxation in HIMA.

As a whole, we could show that endothelial-mediated relaxant responses occur in HIMA. These responses are presumably due to release of EDRF, as defined in rabbit aorta (Furchgott, 1983), that is a non-prostanoid endothelial factor which stimulates the production of cyclic GMP in the vascular smooth muscle; it has recently been proposed that EDRF is in fact NO (Hutchinson *et al.*, 1987).

It is known from bioassay experiments that endothelial cells can spontaneously release EDRF (basal EDRF; Griffith *et al.*, 1984a,b; Rubanyi *et al.*, 1985). Higher levels of cyclic GMP in unrubbed vessels as compared to those in matched rubbed vessels have been ascribed to the effect of this basal EDRF (Holzmann, 1982; Rapoport & Murad, 1983; Diamond & Chu, 1983; Bigaud *et al.*, 1984; Miller *et al.*, 1984; 1985; Martin *et al.*, 1986; Spedding *et al.*,

1986; Bullock *et al.*, 1986; Gruetter & Lemke, 1986b). As a consequence, basal levels of cyclic GMP are regarded as an index of the release of basal EDRF. In HIMA, we found a significant decrease in cyclic GMP levels after rubbing the endothelium. This result indicates that these arteries do liberate EDRF spontaneously. Another indirect evidence for a basal release of EDRF in HIMA can be deduced from the effects of methylene blue in contractile experiments. Methylene blue elicited a slowly developing contraction whose amplitude was significantly greater in unrubbed than in rubbed preparations. Similar observations have been reported in animal arteries (Griffiths *et al.*, 1985; Ignarro *et al.*, 1986). Furthermore methylene blue potentiated the contraction induced by noradrenaline to a greater extent in unrubbed than in rubbed HIMA, still in accordance with other observations (Miller *et al.*, 1984; Griffith *et al.*, 1985; Martin *et al.*, 1985; Murakami *et al.*, 1985; Miyazaki & Toda, 1986). These differences in the effect of methylene blue between unrubbed and rubbed vessels are attributable to the inhibition of the action of basal EDRF on guanylate cyclase. Indomethacin also elicited a slowly developing contraction in HIMA, an effect believed to result from the removal of vasodilator prostanoids (Roberts *et al.*, 1981; Rubanyi & Vanhoutte, 1985); it was similar in unrubbed and rubbed preparations.

The presence of endothelium is known to modulate agonist-induced contractions of animal arteries. This has been particularly shown for noradrenaline (Cocks & Angus, 1983; Fortes *et al.*, 1983; Konishi & Su, 1983; Eglème *et al.*, 1984) and for histamine (Van de Voorde & Leusen, 1983; 1984; Griffith *et al.*, 1984; Miller & Vanhoutte, 1986; Sercombe *et al.*, 1986). In some instances these two agonists have been proved to stimulate EDRF release (Cocks & Angus, 1983; Van de Voorde & Leusen, 1983; Miller & Vanhoutte, 1985). The respective contribution of basal and stimulated EDRF release to endothelial-mediated modulation of contraction has therefore been difficult to estimate. In unrubbed HIMA, we found that the concentration-effect curve of noradrenaline-induced contraction was shifted to the right of that in rubbed HIMA by about two fold. This effect is most probably due to basal EDRF since noradrenaline was unable to elicit an endothelium-dependent relaxation of precontracted HIMA, i.e. to stimulate release of EDRF. Analysis of the concentration-effect curve for histamine-induced contraction of HIMA was somewhat complicated by the fact that the amine triggered rhythmic contractile activity superimposed to increased tonic contraction. This phenomenon, essentially observed in unrubbed preparations, could be partially inhibited by indomethacin and abolished by nifedipine. It is probably due to some histamine-stimulated synthesis of prostaglandin- or thromboxane-like material, as has been shown for example in the rabbit perfused ear (Juan & Sametz, 1980) or in the pulmonary circulation of various animal species (Bakhle & Smith, 1972; Berti *et al.*, 1979). In spite of this rhythmic activity, it was obvious that histamine-elicited contractions were modulated to a greater extent by endothelium than were noradrenaline-induced contractions. In fact, the presence of the endothelium shifted the concentration-effect curve of histamine-induced contraction in unrubbed preparations to the right of those in rubbed preparations by 4 to 9 fold, depending on the level of contraction taken into consideration during rhythmic oscillations triggered by histamine. The greater modulatory effect of endothelium on histamine-elicited contraction might be explained by a basal plus stimulated EDRF release, since histamine, unlike noradrenaline, was able to elicit an endothelium-dependent relaxation of precontracted HIMA. Alternatively, basal EDRF alone could account for the differential modulatory effect of endothelium on agonist-induced contraction of HIMA. Indeed, the presence of endothelium has been associated with an impaired ^{45}Ca influx into some arteries of the rat, rabbit and dog (Collins *et al.*, 1986a,b; Malta *et al.*, 1986b; Godfraind, 1986) and in HIMA histamine-induced contractions were more sensitive to the calcium entry blocker nifedipine than were noradrenaline-induced contractions. Thus, the differential effect of endothelium on histamine- and noradrenaline-induced HIMA contractions may well reflect the different dependence of these contractions of extracellular calcium, in accordance with the proposal that EDRF could affect Ca^{2+} gating into vascular smooth muscle (Godfraind, 1986). Our results confirm those obtained in rat aorta, showing that the more agonist-induced contractions are dependent on extracellular calcium, the more they are inhibited by the endothelium (Eglème *et al.*, 1984; Godfraind *et al.*, 1985).

In the present study, we could thus demonstrate in a human isolated artery that endothelium is not only essential for the vasorelaxation induced by some agents, but that it also modulates contractile responses, probably owing to a spontaneous release of basal EDRF. Such a modulatory effect could be of physiological importance in the regulation of vascular tone, particularly in a vessel used for coronary bypass.

P.S. was partly supported by a grant of the Fondation pour la Recherche Médicale (France). This work has been supported by grant F.R.S.M. n° 3.9006.87 and a grant of the Bekales Foundation.

References

ANGUS, J.A., COCKS, T.M. & SATOH, K. (1986a). α_2-Adrenoceptors and endothelium-dependent relaxation in canine large arteries. *Br. J. Pharmacol.*, **88**, 767–777.

ANGUS, J.A., COCKS, T.M. & SATOH, K. (1986b). The alpha adrenoceptors on endothelial cells. *Fed. Proc.*, **45**, 2355–2359.

ASH, A.S.F. & SCHILD, H.O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmacol. Chemother.*, **27**, 427–439.

BAKHLE, Y.S. & SMITH, T.W. (1972). Release of spasmogenic substances induced by vasoactive amines from isolated lungs. *Br. J. Pharmacol.*, **46**, 543–544P.

BERTI, F., FOLCO, G.C., NICOSIA, S., OMINI, C. & PASARGIKLIAN, R. (1979). The role of histamine H_1- and H_2-receptors in the generation of thromboxane A_2 in perfused guinea-pig lungs. *Br. J. Pharmacol.*, **65**, 629–633.

BIGAUD, M., SCHOEFFTER, P., STOCLET, J.C. & MILLER, R.C. (1984). Dissociation between endothelium-mediated increases in tissue cGMP levels and modulation of aortic contractile responses. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 221–223.

BOSSALLER, C., HABIB, G.B., YAMAMOTO, H., WILLIAMS, C., WELLS, S. & HENRY, P.D. (1986). Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5'-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. *J. Clin. Invest.*, **79**, 170–174.

BULLOCK, G.R., TAYLOR, S.G. & WESTON, A.H. (1986). Influence of the vascular endothelium on agonist-induced contractions and relaxations in rat aorta. *Br. J. Pharmacol.*, **89**, 819–830.

BYFIELD, R.A. & SWANE, G.T.G. (1987). Characterisation of the histamine receptor which mediates EDRF release in a microvascular preparation. *Br. J. Pharmacol.*, **90**, 152P.

CAILLA, H.L., VANNIER, C.J. & DELAAGE, M.A. (1976). Guanosine 3',5'-cyclic monophosphate assay at 10–15-mole level. *Anal. Biochem.*, **70**, 195–202.

CAPLAN, B.A. & SCHWARTZ, C.J. (1973). Increased endothelial cell turnover in areas of in vivo Evans blue uptake in the pig aorta. *Atherosclerosis*, **17**, 401–407.

CARRIER, G.O. & WHITE, R.E. (1985). Enhancement of alpha-1 and alpha-2 adrenergic agonist-induced vasoconstriction by removal of endothelium in rat aorta. *J. Pharmacol. Exp. Ther.*, **232**, 682–687.

CARRIER, G.O., WHITE, R.E. & KIRBY, M.L. (1984). Histamine-induced relaxation of rat aorta. Importance of H_1 receptor and vascular endothelium. *Blood Vessels*, **21**, 180–183.

CHAND, N. & ALTURA, B.M. (1981). Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: role in lung vascular diseases. *Science*, **213**, 1376–1379.

CHERRY, P.D., FURCHGOTT, R.F., ZAWADZKI, J.V. & JOTHIANANDAN, D. (1982). Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 2106–2110.

COCKS, T.M. & ANGUS, J.A. (1983). Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature*, **305**, 627–630.

COLLINS, P., CHAPPELL, S.P., GRIFFITH, T.M., LEWIS, M.J. & HENDERSON, A.H. (1986a). Differences in basal endothelium-derived relaxing factor activity in different artery types. *J. Cardiovasc. Pharmacol.*, **8**, 1158–1162.

COLLINS, P., GRIFFITH, T.M., HENDERSON, H.A. & LEWIS, M.J. (1986b). Endothelium-derived relaxing factor alters calcium fluxes in rabbit aorta: a cyclic guanosine monophosphate-mediated effect. *J. Physiol.*, **381**, 427–437.

DAVIES, J.M. & WILLIAMS, K.I. (1984). Endothelial-dependent relaxant effects of vaso-active intestinal polypeptide and arachidonic acid in rat aortic strips. *Prostaglandins*, **27**, 195–202.

DIAMOND, J. & CHU, E.B. (1983). Possible role for cyclic GMP in endothelium-dependent relaxation of rabbit aorta by acetylcholine. Comparison with nitroglycerin. *Res. Commun. Chem. Path. Pharmacol.*, **41**, 369–381.

EGLEME, C., GODFRAIND, T. & MILLER, R.C. (1984). Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. *Br. J. Pharmacol.*, **81**, 16–18.

FORSTERMANN, U., HERTTING, G. & NEUFANG, B. (1986a). The role of endothelial and non-endothelial prostaglandins in the relaxation of isolated blood vessels of the rabbit induced by acetylcholine and bradykinin. *Br. J. Pharmacol.*, **87**, 521–532.

FORSTERMANN, U., MUGGE, A. & FROLICH, J.C. (1986b). Endothelium-dependent relaxation of human epicardial coronary arteries: frequent lack of effect of acetylcholine. *Eur. J. Pharmacol.*, **128**, 227–281.

FORTES, Z.B., GARCIA LEME, J. & SCIVOLETTO, R. (1983). Vascular reactivity in diabetes mellitus: role of the endothelial cell. *Br. J. Pharmacol.*, **79**, 771–781.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines*, ed. Blaschko, H. & Muscholl, E. pp. 283–335. Berlin: Springer-Verlag.

FURCHGOTT, R.F. (1981). The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. *Trends Pharmacol. Sci.*, **2**, 173–176.

FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circ. Res.*, **53**, 557–573.

FURCHGOTT, R.F. (1984). The role of endothelium in the responses of vascular smooth muscle to drugs. *Ann. Rev. Pharmacol. Toxicol.*, **24**, 175–197.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

GINSBURG, R. & ZERA, P.H. (1984). Endothelial relaxant factor in the human epicardial coronary artery. *Circulation*, **70**, suppl. II, 122.

GODFRAIND, T. (1982). Actions of nifedipine on calcium fluxes and contraction in isolated rat arteries. *J. Pharmacol. Exp. Ther.*, **224**, 443–450.

GODFRAIND, T. (1986). EDRF and cyclic GMP control gating of receptor-operated calcium channels in vascular smooth muscle. *Eur. J. Pharmacol.*, **126**, 341–343.

GODFRAIND, T., EGLEME, C. & AL OSACHIE, I. (1985). Role of endothelium in the contractile response of rat

aorta to alpha-adrenoceptor agonists. *Clin. Sci.*, **68**, suppl. 10, 65s–71s.

GORDON, J.L. & MARTIN, W. (1983). Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of 86Rb efflux from isolated endothelial cells. *Br. J. Pharmacol.*, **79**, 531–541.

GRIFFITH, T.M., EDWARDS, D.H., LEWIS, M.J. & HENDERSON, A.H. (1985). Evidence that cyclic guanosine monophosphate (cGMP) mediates endothelium-dependent relaxation. *Eur. J. Pharmacol.* **112**, 195–202.

GRIFFITH, T.M., EDWARDS, D.H., LEWIS, M.J., NEWBY, A.C. & HENDERSON, A.H. (1984a). The nature of endothelium-derived vascular relaxant factor. *Nature*, **308**, 645–647.

GRIFFITH, T.M., HENDERSON, A.H., EDWARDS, D.H. & LEWIS, M.J. (1984b). Isolated perfused rabbit coronary artery and aortic strip preparations: the role of endothelium-derived relaxant factor. *J. Physiol.*, **351**, 13–24.

GRUETTER, C.A. & LEMKE, S.M. (1986a). Bradykinin-induced endothelium-dependent relaxation of bovine intrapulmonary artery and vein. *Eur. J. Pharmacol.*, **122**, 363–367.

GRUETTER, C.A. & LEMKE, S.M. (1986b). Comparison of endothelium-dependent relaxation in bovine intrapulmonary artery and vein by acetylcholine and A23187. *J. Pharmacol. Exp. Ther.*, **238**, 1055–1062.

HOLZMANN, S. (1982). Endothelium-induced relaxation by acetylcholine associated with larger rises in cyclic GMP in coronary arterial strips. *J. Cyclic Nucleotide Res.*, **8**, 409–419.

HUTCHINSON, P.J.A., PALMER, R.M.J. & MONCADA, S. (1987). Comparative pharmacology of EDRF and nitric oxide on vascular strips. *Eur. J. Pharmacol.*, **141**, 445–451.

IGNARRO, L.J., HARBISON, R.G., WOOD, K.S. & KADOWITZ, P.J. (1986). Dissimilarities between methylene blue and cyanide on relaxation and cyclic GMP formation in endothelium-intact intrapulmonary artery caused by nitrogen oxide-containing vasodilators and acetylcholine. *J. Pharmacol. Exp. Ther.*, **236**, 30–36.

JUAN, H. & SAMETZ, W. (1980). Histamine-induced release of arachidonic acid and of prostaglandins in the peripheral vascular bed. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **314**, 183–190.

KONISHI, M. & SU, C. (1983). Role of endothelium in dilator responses of spontaneously hypertensive rat arteries. *Hypertension*, **5**, 881–886.

LEUSEN, I. & VAN DE VOORDE, J. (1986). Histamine-induce release of endothelium-derived relaxing factor from human umbilical blood vessels. *Blood Vessels*, **23**, 86.

LOOP, F.D., LYTLE, B.W., COSGROVE, D.M., STEWART, R.W., GOORMASTIC, M., WILLIAMS, G.W., GOLDING, L.A.R., GILL, C.C., TAYLOR, P.C., SHELDON, W.C. & PROUDFIT, W.L. (1986). Influence of the internal-mammary-artery graft on 10-year survival and other cardiac events. *New England J. Med.*, **314**, 1–6.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.L. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

LUES, I. & SCHUMANN, H.J. (1984). Effect of removing the endothelial cells on the reactivity of rat aortic segments to different alpha-adrenoceptor agonists. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 160–163.

MALTA, E., SCHINI, V. & MILLER, R.C. (1986a). Role of efficacy in the assessment of the actions of alpha-adrenoceptor agonists in rat aorta with endothelium. *J. Pharm. Pharmacol.*, **38**, 209–213.

MALTA, E., SCHINI, V. & MILLER, R.C. (1986b). Effect of endothelium on basal and alpha-adrenoceptor stimulated calcium fluxes in rat aorta. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **334**, 63–70.

MARTIN, W., FURCHGOTT, R.F., VILLANI, G.M. & JOTHIANANDAN, D. (1986). Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther.*, **237**, 529–538.

MARTIN, W., VILLANI, G.M., JOTHIANDAN, D. & FURCHGOTT, R.F. (1985). Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J. Pharmacol. Exp. Ther.*, **232**, 708–716.

MILLER, R.C., MONY, M.C., SCHINI, V., SCHOEFFTER, P. & STOCLET, J.C. (1984). Endothelial mediated inhibition of contraction and increase in cyclic GMP levels evoked by the alpha-adrenoceptor agonist B-HT 920 in rat isolated aorta. *Br. J. Pharmacol.*, **83**, 903–908.

MILLER, R.C., SCHOEFFTER, P. & STOCLET, J.C. (1985). Insensitivity of calcium-dependent endothelial stimulation in rat isolated aorta to the calcium entry blocker, flunarizine. *Br. J. Pharmacol.*, **85**, 481–487.

MILLER, V.M. & VANHOUTTE, P.M. (1985). Endothelial $alpha_2$ adrenoceptors in canine pulmonary and systemic blood vessels. *Eur. J. Pharmacol.*, **118**, 123–129.

MILLER, V.M. & VANHOUTTE, P.M. (1986). Endothelium-dependent responses in isolated blood vessels of lower vertebrates. *Blood Vessels*, **23**, 225–235.

MIYAZAKI, M. & TODA, N. (1986). Endothelium-dependent changes in the response to vasoconstrictor substances of isolated dog mesenteric veins. *Jap. J. Pharmacol.*, **42**, 309–316.

MURAKAMI, K., KARAKI, H. & URAKAWA, N. (1985). Role of endothelium in the contractions induced by norepinephrine and clonidine in rat aorta. *Jap. J. Pharmacol.*, **39**, 357–364.

RAPOPORT, R.M. & MURAD, F. (1983). Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ. Res.*, **52**, 352–357.

ROBERTS, A.M., MESSINA, E.J. & KALEY, G. (1981). Prostacyclin (PGI_2) mediates hypoxic relaxation of bovine coronary arterial strips. *Prostaglandins*, **21**, 555–569.

RUBANYI, G.M., LORENZ, R.R. & VANHOUTTE, P.M. (1985). Bioassay of endothelium-derived relaxing factor(s): inactivation by catecholamines. *Am. J. Physiol.*, **249**, H95–H101.

RUBANYI, G. & VANHOUTTE, P.M. (1985). Endothelium-removal decreases relaxations of canine coronary arteries caused by adrenergic agonists and adenosine. *J. Cardiovasc. Pharmacol.*, **7**, 139–144.

SATOH, H. & INUI, J. (1984). Endothelial cell-dependent relaxation and contraction induced by histamine in the isolated guinea-pig pulmonary artery. *Eur. J. Pharmacol.*, **97**, 321–324.

SERCOMBE, R., VERRECCHIA, C., PHILIPSON, V., OUDART, N., DIMITRIADOU, V., BOUCHAUD, C. & SEYLAZ, J. (1986). Histamine-induced constriction and dilatation of rabbit middle cerebral arteries in vitro: role of the endothelium. *Blood Vessels*, **23**, 137–153.

SINGER, H.A. & PEACH, M.J. (1983). Endothelium-dependent relaxation of rabbit aorta. II. Inhibition of relaxation stimulated by methacholine and A23187 with antagonists of arachidonic acid metabolism. *J. Pharmacol. Exp. Ther.*, **226**, 796–801.

SPEDDING, M., SCHINI, V., SCHOEFFTER, P. & MILLER, R.C. (1986). Calcium channel activation does not increase release of endothelial-derived relaxant factors (EDRF) in rat aorta although tonic release of EDRF may modulate calcium channel activity in smooth muscle. *J. Cardiovasc. Pharmacol.*, **8**, 1130–1137.

THOM, S.A., HUGHES, A.D., MARTIN, G.N. & SEVER, P.S. (1985). The release of endothelium derived relaxing factor from isolated human arteries. *J. Hypertension*, **3**, suppl. 3, S97–S99.

THOM, S., HUGHES, A.D., MARTIN, G. & SEVER, P. (1986). In vitro pharmacological responses of human coronary arteries. *Blood Vessels*, **23**, 102.

TODA, N. (1986). Mechanisms of histamine-induced relaxation in isolated monkey and dog coronary arteries. *J. Pharmacol. Exp. Ther.*, **239**, 529–535.

VAN DE VOORDE, J. & LEUSEN, I. (1983). Role of endothelium in the vasodilator response of rat thoracic aorta to histamine. *Eur. J. Pharmacol.*, **87**, 113–120.

VAN DE VOORDE, J. & LEUSEN, I. (1984). Effect of histamine on aorta preparations of different species. *Arch. Int. Pharmacodyn. Ther.*, **268**, 95–105.

VANHOUTTE, P.M., RUBANYI, G.M., MILLER, V.M. & HOUSTON, D.S. (1986). Modulation of vascular smooth muscle contraction by the endothelium. *Ann. Rev. Physiol.*, **48**, 307–320.

WHALLEY, E.T., AMURE, Y.O. & LYE, R.H. (1987). Analysis of the mechanism of action of bradykinin on human basilar artery in vitro. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **335**, 433–437.

ZAWADZKI, J.V., CHERRY, P.D. & FURCHGOTT, R.F. (1980). Comparison of endothelium-dependent relaxation of rabbit aorta by A23187 and by acetylcholine. *Pharmacologist*, **22**, 271.

(Received October 28, 1987
Accepted May 9, 1988)