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Quantitative changes in pharmacodynamic parameters of noradrenaline in different rat aorta preparations: influence of endogenous EDRF

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Summary

1 The aim of the present study was to assess the role of endothelial cells in the modulation of vasocontractile responses to noradrenaline in rat isolated aorta when cut as standard helical strips or as ring segments.

2 Noradrenaline-potency in helical strip preparations evaluated as –logEC50 was greater than that obtained in endothelium-intact ring preparations (9.45 ± 0.28 versus 8.69 ± 0.09, respectively) ($P < 0.05$). The maximum contractile response of helical strips was significantly higher than the response of ring preparations ($P < 0.05$).

3 Subsequent experiments were performed on helical strips and ring preparations where the endothelium was removed by rubbing the luminal surface of the aorta with filter paper. Removal of the endothelium potentiated the noradrenaline-induced contraction in ring preparations, but not in the helical strips.

4 The nitric oxide synthase inhibitors L-NAME (3×10^{-5}–3×10^{-4} M) or L-NNA (1×10^{-4}–3×10^{-4} M) which were added to the tissue bath potentiated the noradrenaline-induced contraction in the endothelium-intact ring preparations, although only L-NNA induced a statistically significant potentiation. Both L-NAME and L-NNA had no effect on the noradrenaline-contraction induced in rings without endothelium, or in helical strips with or without endothelium.

5 Vascular acetylcholine-induced relaxation is dependent on endothelium derived relaxing factor (nitric oxide). Acetylcholine (10^{-9}–10^{-6} M) induced a concentration-dependent relaxation in noradrenaline preconstricted intact rings. The relaxant response was strongly reduced by L-NAME (3×10^{-5}–1×10^{-4} M). The relaxant response to acetylcholine was very weak in ring and helical strip preparations without endothelium, but also, surprisingly, in unrubbed standard helical strips.

6 The present results suggest that the endothelium of standard helical strip preparations may be greatly damaged, a view confirmed by morphological studies. The structural and functional damage of the endothelium induced very important changes in pharmacodynamic parameters such as in the potency and the maximal responses of vascular preparations to noradrenaline. Therefore, caution must be observed when the potency and intrinsic activity of agonists evaluated on different preparations are compared, even if these come from the same vascular segment.

Introduction

The vascular endothelium represents a key element in regulating vascular reactivity in physiopathological conditions, and in vascular responses to endogenous or exogenous substances. In fact, vascular muscle tone is modulated by substances released by endothelial cells, such as prostacycline (Moncada, Herman, Higgs, & Vane, 1977), endothelium-derived relaxing factor (EDRF) (Furchgott & Zawadzki, 1980), endothelium-derived hyperpolarizing factor (EDHF) (Taylor & Weston, 1988; Zygmunt & Hogestatt, 1996) and contracting factors including endothelins (Yanagisawa *et al.*, 1988; Luscher, Boulanger, Dohi & Yang, 1992). When studying vasoactive substances or vascular reactivity in physiopathological conditions, a major concern in the field is whether *in vitro* data are predictive of *in vivo* activity and how comparable results are among different laboratories. Given that the best correlation between results obtained *in vitro* and *in vivo* is a situation where the experimental model exactly reproduces physiological conditions, the use of a vascular tissue prepared without its endothelium could be criticized as having little

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predictive value. Indeed, some inconsistencies in data published by different groups (Beckeringh *et al.*, 1984; Macia, Matthews, Lafferty & Demarinis, 1984; Alosachie & Godfraind, 1986) may derive from the fact that vascular preparations, even of the same segments, differ between laboratories; for example, vessels prepared as helical strips, according to the classical method suggested by Furchgott & Badrakom (1953), versus vessels prepared as rings. In addition, each of these preparations can be prepared with or without endothelium. In spite of these considerations, there is a lack of studies that have directly and systematically assessed the effect of the preparation type and experimental setting on the activity of vasoactive substances. Accordingly, the aim of the present work was to study the reactivity to noradrenaline, a standard substance in studies on the characterization of α-adrenoceptors in rat isolated aorta when prepared either as helical strips or as ring segments. Both direct responses to this agonist and modulatory effects on its contracting activity were studied. Comparative studies were carried out on tissues with or without an intact endothelium to assess the role of the endothelium in the contractile responses.

Methods

Isolated aortic preparations

Male Wistar rats (Morini, Italy) weighing 300–350 g were anaesthetized with CO_2 and exsanguinated from the common carotid arteries. A section of the thoracic aorta between the aortic arch and diaphragm was quickly removed and placed in a modified Krebs–Henseleit solution (KHS). The composition of the modified KHS was as follows (mM): NaCl, 118; KCl, 5.6; $CaCl_2$, 2.5; $MgSO_4$, 1.19; NaH_2PO4, 1.3; $NaHCO_3$, 25; glucose, 10; Na_2EDTA, 3.7×10^{-3}, to prevent the oxidative degradation of noradrenaline; propranolol, 1×10^{-4}, to inhibit β-adrenoceptor activity; cocaine, 1×10^{-3}, to block neuronal uptake; and hydrocortisone, 2×10^{-3}, to block extraneuronal uptake. The vessel was carefully cleaned of adherent fat and connective tissue. Intraluminal blood was removed by gentle lavage in KHS. Using the same artery, a transverse ring of 5 mm width was cut with scissors or a standard helical strip was made by cutting the arterial wall across a ring of the same width as described by Furchgott & Badrakom (1953); special care was taken to avoid unintentional rubbing of the intimal surface in both preparations.

In some experiments, the luminal surface of the helical and ring segments was rubbed with filter paper to remove the endothelium (Furchgott & Zawadzki, 1980). The helical strips and rings were then suspended between two wire hooks, with one wire attached to a fixed tissue support in an isolated tissue bath containing KHS maintained at 37 °C, and gassed with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). The other wire was connected by a silk thread to a Grass FT.03 force displacement transducer attached to a Battaglia Rangoni polygraph.

Experimental protocol

The preparations were placed under a resting tension of 2 g, a tension previously found to be optimal for this tissue (F. Zonta, unpublished data). The KHS in the bath was replaced every 20 min for a 90 min equilibration period and then the basal tone of the equilibrated aortic preparations was evaluated by replacing the KHS with Ca^{2+}-free KHS containing 2-mM EGTA. The decrease in force (relaxation) over a period of 30–40 min of washing in Ca^{2+}-free solution was used as a measure of the inherent tone in the preparation.

Each preparation in normal KHS was exposed to noradrenaline (3×10^{-8} M) and allowed to contract for 5 min. Following complete washout, the preparation was again challenged with noradrenaline (3×10^{-8} M) and washed, and an additional 1 h equilibration period was allowed before starting the experiment. This procedure minimized changes in the sensitivity of the preparation to further addition of agonists. Cumulative concentration–response curves (CRCs) to noradrenaline (1×10^{-11}–1×10^{-7} M) were determined in each preparation by plotting the contractile response of the tissue to increasing concentrations (0.5 log unit increments). Each concentration of noradrenaline was left in the bath for 1–3 min to reach a plateau response.

Cumulative concentration-response curves to acetylcholine (10^{-9}–3×10^{-6} M) were constructed in both endothelium-containing preparations and denuded preparations following a submaximal noradrenaline (3×10^{-8} M)-induced tone. Successive CRCs to agonists were made at 60 min intervals, as measured from the time following washout required to obtain the basal tone.

In another set of experiments, indomethacin (1×10^{-5} M) or a nitric oxide synthase inhibitor, L-NAME (3×10^{-5} M–3×10^{-4} M) or L-NNA (1×10^{-4}, 3×10^{-4} M), were incubated for 30 min in a bath containing KHS. Following the incubation period, concentration-response curves to noradrenaline or acetylcholine were measured in the presence of inhibitors.

Morphological examination

A set of pharmacological experiments was followed by treatment for morphological study.

Samples of aorta wall were immediately immersed in the fixative solution (2% paraformaldehyde–2.5% glutaraldehyde Na-cacodylate buffered at pH 7.4) and fixation was continued for 6 h. Postfixation with 1.33% OsO_4 in s-collidine buffer was also performed, and after dehydration in a graded ethanol, specimens were embedded in Epon 812 epoxy resin; flat embedding was done in order to obtained the best

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orientation of sections, i.e. transversal to the arterial wall. Semithin (0.2–1 μm) and ultrathin (20–50 nm) sections were made with an ultramicrotome (Reichert Ultracut E) and stained for light (toluidine blue) and electron microscope (uranyl acetate and lead citrate) examination. Observations and micrographs were made with a Zeiss EM 10 transmission electron microscope operating at 80 kV with an objective aperture of 50 μm.

Analysis of data

At the end of each experiment, aortic preparations were blotted dry, measured and weighed. The cross-sectional area of each preparation was calculated using the formula: cross-sectional area (mm^2) = weight (mg)/length (mm) × density (mg mm^{-3}), where the density was assumed to be 1.05 mg mm^{-3} (Wyse, 1980). Contractile responses of each preparation to noradrenaline were calculated as the increase in tension (g) in response to each concentration of the drug per cross-sectional area of the tissue to give values in g mm^{-2}. Relaxations to acetylcholine were expressed as the percentage reversal of the noradrenaline-induced contraction.

The negative log EC_{50} values, where EC_{50} was the molar concentration producing 50% of the maximum agonist effect (E_{max}), were calculated from cumulative concentration–response curves by linear regression analysis of data points from individual concentration–response curves (Tallarida & Murray, 1981).

The –log EC_{50} values were used to assess the sensitivity of tissue to agonists. Potency of L-NAME or L-NNA was evaluated by reduction of acetylcholine-induced maximum relaxation (E_{max}) or by the potentiating effect of noradrenaline-induced contraction.

The results were expressed as means ± SE; *n* refers to the number of experiments. One-way analysis of variance (ANOVA) was used when more than two groups were analysed.

Statistical differences between two means were determined by Student's *t*-test for paired or unpaired observations where appropriate. A value of $P < 0.05$ was considered to be statistically significant.

Drugs

(–)-Noradrenaline-L-hydrochloride, acetylcholine chloride, ethylene glycol-bis (beta-amino-ethyl ether) N,N,N,N,-tetraacetic acid (EGTA), indomethacin, N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-L-arginine (L-NNA) were obtained from Sigma (St Louis, MO, USA). Stock solution of noradrenaline (10^{-2} M) was prepared in the presence of 30 mg ml^{-1} ascorbic acid to prevent oxidation of the drug. Indomethacin was dissolved in distilled water. Prior to each experiment, an aliquot of each drug was diluted with distilled water to the appropriate concentration.

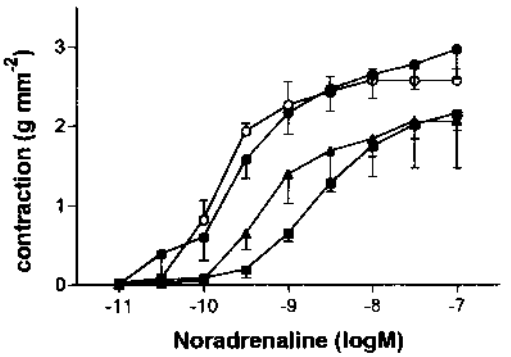

Figure 1 Concentration-response curves to noradrenaline in the rat aorta preparations: (●) unrubbed and (○) rubbed helical strips, and (■) unrubbed and (▲) rubbed ring preparations. Each point is the mean ± SE of six to eight observations.

Results

Contractile response to noradrenaline

The cumulative addition of noradrenaline contracted all aortic standard helical strips and rings in a concentration-dependent manner (Fig. 1). The potency of noradrenaline in the helical strips with unrubbed endothelium (expressed as $-\log EC_{50}$) was 9.45 ± 0.28; the magnitude of the maximum contractile response (E_{max}) was 2.96 ± 0.37 g mm^{-2}; no change of noradrenaline potency (9.65 ± 0.07) or maximum contractile response (2.57 ± 0.15 g mm^{-2}) was observed in the endothelium-denuded helical strips (Table 1).

The potency of noradrenaline in the endothelium-intact ring (8.69 ± 0.09) was significantly ($P < 0.05$) lower than that measured in the standard helical strips. Also, the magnitude of the maximum contractile response was decreased (2.15 ± 0.21 g mm^{-2}). Removal of the endothelium in the ring preparations significantly increased the potency of noradrenaline (9.36 ± 0.07), without changing the maximum response (2.06 ± 0.59 g mm^{-2}), which was signifi-

Table 1 Potencies (mean negative $\log EC_{50}$ values) and maximal contractile responses (E_{max}) of noradrenaline in the rat aorta preparations[a]

Preparations	$-\log EC_{50}$	E_{max} (g mm^{-2})
Unrubbed helical strips	9.45 ± 0.28	2.96 ± 0.37
Rubbed helical strips	9.65 ± 0.07	2.57 ± 0.15
Unrubbed rings	8.69 ± 0.09[b]	2.15 ± 0.21[c]
Rubbed rings	9.36 ± 0.07	2.06 ± 0.59[d]

[a]All data are expressed as means ± SE (n = 6–8); n = number of experiments.
[b]Significantly different from unrubbed helical strips and rubbed preparations ($P < 0.01$).
[c]Significantly different from unrubbed helical strips ($P < 0.05$).
[d]Significantly different from rubbed helical strips ($P < 0.05$).

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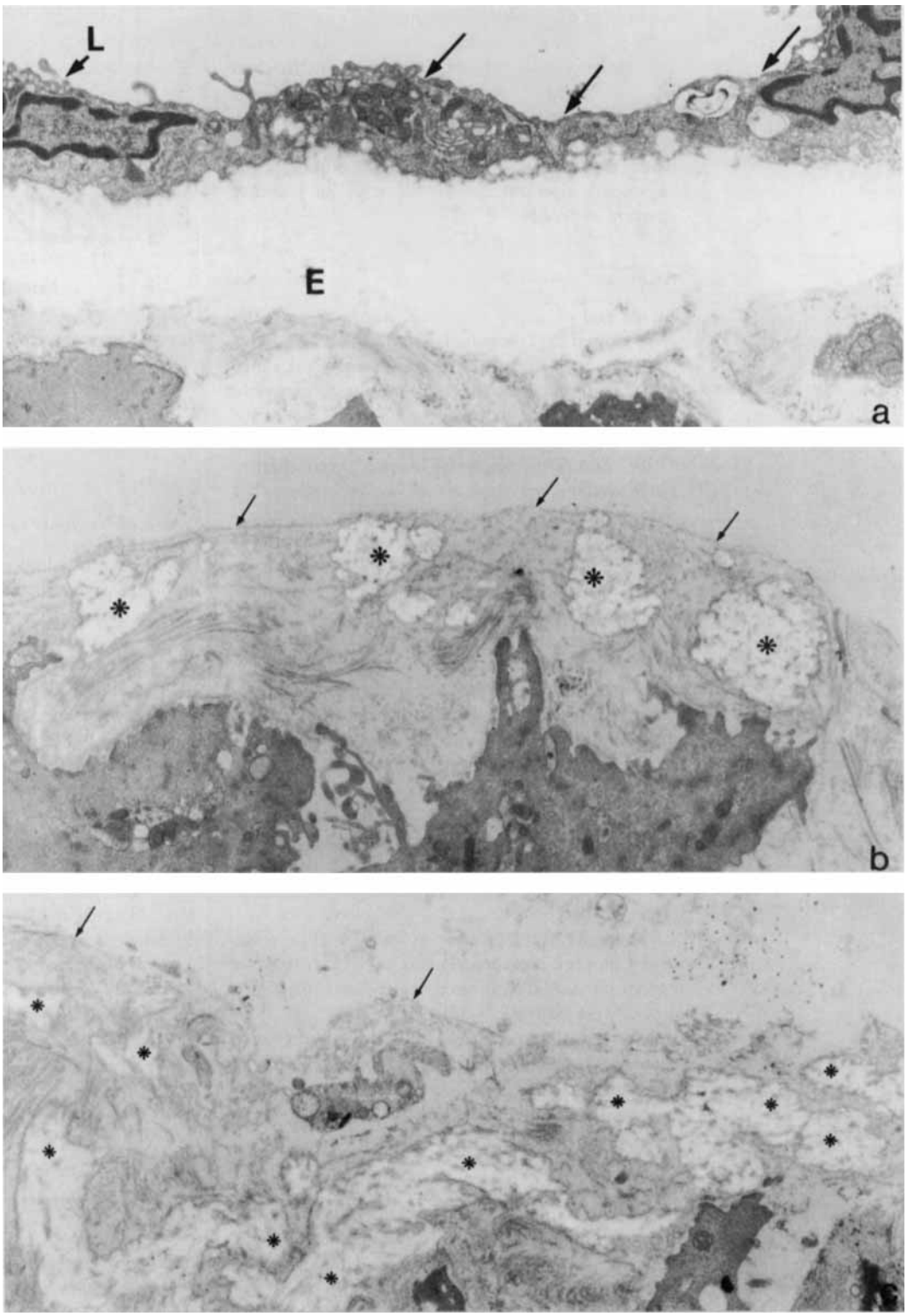

Figure 2 (a) Unrubbed transverse ring of aorta. The endothelial lining is continuous (arrows) and the ultrastructural preservation of endothelial cells is good: (L) lumen of the vessel; and (E) elastic lamina (electron micrograph, ×15 500). (b) Unrubbed helical strip of aorta. The endothelial lining is lacking and basement membrane remnant is visible (arrows); elastic fibres are arranged in separated bundles (asterisks) (electron micrograph, ×15 500). (c) Rubbed transverse ring of aorta. The endothelial lining is lacking and small basement membrane remnants are visible (arrows); elastic fibres are diversely oriented and show a frayed appearance (asterisks) (electron micrograph, ×15 500).

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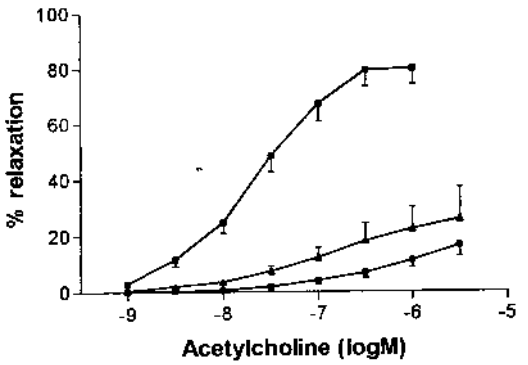

Figure 3 Concentration-response curves to acetylcholine on noradrenaline (3×10^{-8} M)-contracted rat aorta preparations: (▲) unrubbed helical strips, and (■) unrubbed and (●) rubbed ring preparations. Each point is the mean ± SE of six to ten observations.

cantly lower than that obtained in the helical strips. The potencies ($-\log EC_{50}$) and maximum contractile effects (E_{max}) are summarized in Table 1.

Effects of Ca^{2+}-free Krebs–Henseleit solution containing EGTA on aortic preparations

Replacement of the Krebs–Henseleit solution with Ca^{2+}-free Krebs–Henseleit solution containing EGTA (2 mM) had no effect on the resting force of either helical strips or the rings with or without endothelium (data not shown). This suggested that these preparations were already fully relaxed, i.e. they had no inherent tone.

Morphological findings

Three preparations of the arterial wall were examined: (1) transverse ring (standard or unrubbed); (2) helical strip (standard or unrubbed); and (3) transverse ring with luminal surface rubbed, as described in the 'Methods' section.

In the preparations of unrubbed transverse ring, the endothelial lining of the arterial wall appeared to be continuous and endothelial cells were well preserved; elastic fibres constituted a continuous lamina below the endothelium (Fig. 2a).

In the vascular wall of helical strips, the endothelium was lacking and remnants of endothelial basement membrane appeared to be directly exposed on the luminal surface of the aorta; a network of collagen fibres and separated bundles of elastic fibres being the prominent structures of the inner part of the arterial wall (Fig. 2b).

In the rubbed preparations of transverse rings, the endothelium was lacking and small remnants of basement membrane were recognizable on the luminal surface of the vessel; many diversely oriented elastic fibres with a frayed appearance, intermingled with irregularly oriented collagen fibres, were detectable (Fig. 2c).

The experimental evidence showing the absence of endothelium in the unrubbed helical strips obviously made the morphological examination of the rubbed preparation unnecessary.

Responses to acetylcholine on noradrenaline-precontracted aortic preparations

Standard helical strips and ring preparations with or without endothelium contracted in response to the submaximal concentration of noradrenaline (3×10^{-8} M). The contraction remained at a stable peak tension for at least 30 min.

The relaxant response to acetylcholine (10^{-9}–3×10^{-6} M) in the precontracted standard helical strips was very weak: maximal relaxation was 16.5 ± 3.8%, the $-\log EC_{50}$ value was not evaluated owing to the negligible relaxation effect (Fig. 3).

The acetylcholine response in rubbed helical strips was similar to that seen on standard unrubbed helical strips (data not shown).

Acetylcholine caused a marked concentration-dependent relaxation of endothelium-intact ring preparations; the potency value was 7.59 ± 0.08 and maximal relaxation was 80.0 ± 5.6%. The removal of the vascular endothelium almost totally inhibited the ability of acetylcholine to relax the ring preparation: maximal relaxation was 25.7 ± 11.5%; the $-\log EC_{50}$ value was not determined owing to the weak relaxation response. The potencies and maximal

Table 2 Potencies (mean negative $\log EC_{50}$ values) and maximal relaxant responses (E_{max}) of acetylcholine in the absence (control) and presence of L-NAME (1×10^{-4} M) in noradrenaline (3×10^{-8} M)-precontracted unrubbed helical strips and ring preparations[a]

	Acetylcholine (control)		Acetylcholine + L-NAME	
Preparations	$-\log EC_{50}$	E_{max} (% reversal of NA contraction)	$-\log EC_{50}$	E_{max} (% reversal of NA contraction)
Unrubbed helical strips	ND	16.5 ± 3.8[b]	ND	ND
Unrubbed rings	7.59 ± 0.08	80.0 ± 5.6	6.57 ± 0.08	47.8 ± 15[c]

[a]All data are expressed as mean ± SEM (n = 6–10); (ND) not determined because of negligible acetylcholine-induced relaxation.
[b]Value significantly less than in unrubbed ring preparations ($P < 0.01$).
[c]Value significantly less than respective value in the absence of L-NAME ($P < 0.01$).

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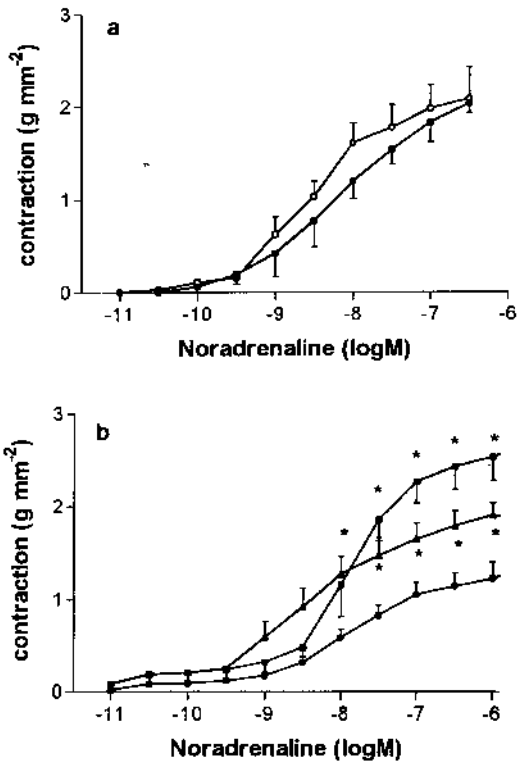

Figure 4 Concentration-response curves to noradrenaline in unrubbed ring preparations of the rat aorta: (a) in the absence (●) and presence (○) of L-NAME (1×10^{-4} M); (b) in the absence (●) and presence of L-NNA (■, 1×10^{-4} M; and ▲, 3×10^{-4} M). Each point is the mean ± SE of eight to ten observations. Asterisks indicate significant differences from respective values in the absence of L-NNA (●); $P < 0.05$.

relaxations measured in unrubbed aortic preparations are summarized in Table 2.

Effects of indomethacin, L-NAME and L-NNA on responses to noradrenaline and acetylcholine

Indomethacin was used to investigate the involvement of prostanoids in the changes in basal and agonist-induced muscle tone. At a concentration of 1×10^{-5} M, indomethacin was inactive both on basal and noradrenaline-induced active tone and on relaxant responses induced by acetylcholine (data not shown).

To investigate whether NO might be the endothelium-derived substance modulating the basal tone, experiments were carried out in which the generation of NO was inhibited by L-NAME or L-NNA. These compounds at concentrations of 3×10^{-5}–3×10^{-4} M had a negligible effect on the basal tone of all types of aortic preparations (data not shown).

To investigate whether NO might be released from the endothelium when the tone was acutely increased by a spasmogen, L-NAME or L-NNA (3×10^{-5}–3×10^{-4} M) was included in KHS 30 min before noradrenaline. L-NAME had a negligible effect on the noradrenaline-induced active tone in rubbed and unrubbed helical strips, and in rubbed ring preparations. This compound potentiated the noradrenaline-induced contraction in unrubbed ring preparation, but this potentiation did not reach statistical significance (Fig. 4a). L-NNA was able to induce a very significant potentiation of the noradrenaline-induced contraction in unrubbed ring preparations of the rat aorta (Fig. 4b).

To investigate whether acetylcholine-induced relaxation evoked endothelial NO biosynthesis and release, experiments were carried out to evaluate the effect of L-NAME on responses to acetylcholine. Since the acetylcholine-induced relaxation was negligible in standard helical strips and in preparations from which the endothelium had been removed, the present authors only studied the effect of L-NAME in the unrubbed ring preparations. The acetylcholine-induced relaxation was greatly inhibited by the pretreatment of the intact-ring with L-NAME (3×10^{-5} M); higher concentrations of L-NAME (1×10^{-4} M) did not increase this antagonism of the relaxant effect of acetylcholine. The concentration–response curve of acetylcholine made in the presence of the inhibitor was flattened. The potency value and the maximal relaxation were 6.57 ± 0.08 and 47.8 ± 15%, respectively, compared to 7.59 ± 0.08 and 80.0 ± 5.6% obtained in the absence of the L-NAME (Fig. 5, Table 2).

The comparison of E_{max} values for acetylcholine obtained in the absence and in the presence of L-NAME showed that the relaxation-response was greatly reduced by the inhibition of NO synthase, but L-NAME showed a less marked effect as compared to endothelial removal (maximum relaxation: 47.8 ± 15% and 25.7 ± 11.5%, respectively) (Table 2, Fig. 3). These data indicate that relaxation might be dependent on the endothelium and mediated by EDRF (NO); the L-NAME-resistant relaxation response to acetylcholine is most likely mediated by another factor (see 'Discussion').

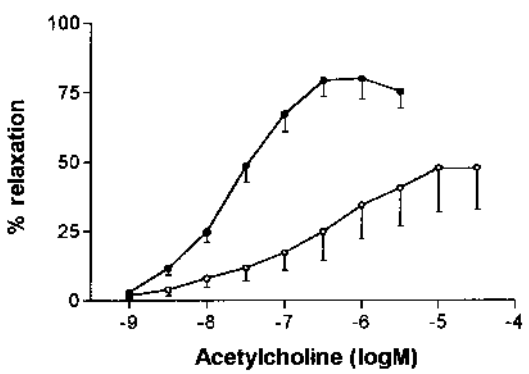

Figure 5 Concentration-response curves to acetylcholine in noradrenaline (3×10^{-8} M)-contracted unrubbed ring preparations in the absence (●) and presence (○) of L-NAME (1×10^{-4} M) applied 30 min prior to the concentration-response experiment. Each point is the mean ± SE of six to eight observations.

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Discussion

The results obtained in the present study clearly demonstrate differences in responses to noradrenaline in the rat aorta when prepared as a standard helical strip following all the precautions suggested by Furchgott & Badrakom (1953) or when prepared as a ring (Furchgott & Zawadzki, 1980). In fact, the noradrenaline potency in the standard helical strip preparations was 10 times higher than that seen in the rings. Even the maximum effect of noradrenaline (1×10^{-7} M) showed an higher response in the standard helical strips when compared to the ring preparations; this might be because of a partial contraction of the ring preparation as a result of inherent tone. Assessment of the inherent tone, as quantified by measuring the relaxation (from resting baseline) induced by bathing the preparations in Ca^{2+}-free physiological salt solution containing 2 mM EGTA, indicated that there was no effect on the resting base-line in either preparation, confirming recent observations on rat pulmonary artery (Wanstall, Hughes & O'Donnell, 1995) and indicating that these preparations were already fully relaxed with no inherent tone. Therefore, the increased maximum noradrenaline effect in standard helical strips is probably caused by a more effective involvement of longitudinal fibres as compared to the ring preparation.

Comparative experiments were performed on helical strips and ring preparations whose endothelium was removed by rubbing the luminal surface of the aorta with filter paper. It is known that removal of the endothelium can potentiate the responses of vascular smooth muscle to various vasoactive agents in certain blood vessels (Randall, Kay & Hiley, 1988; MacLean, McCulloch & MacGrath, 1993; Ortiz *et al.*, 1995). In addition, it has been shown in numerous blood vessels, that the endothelium-dependent modulation of agonist-induced contractile responses is related to the release of relaxing factors from the endothelium, which, in turn, result in a reduction of agonist potency and efficacy (Alosachie & Godfraind, 1986, 1988; Pipili-Synetos, Sideri & Maragoudakis, 1991; Adeagbo & Triggle, 1993).

The present data demonstrate that noradrenaline produced concentration-dependent contractions on the endothelium-free rings. Its potency was approximately five times greater than that obtained in the intact rings. The maximum response to noradrenaline was unaffected by endothelium removal. The potency of noradrenaline in the helical strips without endothelium was the same as that observed in the standard helical strips. When examined by morphological and ultrastructural studies, the preparations that had the intimal surface rubbed on filter paper appeared damaged by the rubbing procedure: the surface was devoid of endothelial cells. Therefore, the present authors are confident that this rubbing procedure produced maximum disruption of the vascular endothelium. In the unrubbed ring preparations, scanning electron microscopy revealed that the luminal surface was completely covered with endothelial cells, regularly arranged parallel to the direction of the blood stream. On the other hand, morphological analysis of the unrubbed helical strips revealed that the endothelium was almost absent. Therefore, it must be stressed that, when cut helically, the aorta is a preparation with a disrupted endothelium. These findings led the present authors to carry out experiments with pharmacological tools that demonstrate an impairment of the functional integrity of the endothelium. It is well known that arterial endothelial cells modulate vascular tone through the production of endothelium-derived relaxing or contracting factors (EDRF or EDCF). Acetylcholine causes relaxation of vascular smooth muscle cells in the presence of active tone by releasing an endothelium-derived relaxing factor in a variety of blood vessels (Furchgott & Zawadzky, 1980; Furchgott, 1983; Altiere, Kiritsy-Roy & Catravas, 1986). Therefore, the present authors tested the acetylcholine response in both intact and endothelium-removed preparations. In intact rings, acetylcholine produced a concentration-dependent relaxation of the noradrenaline-precontracted preparation; the acetylcholine-induced relaxation was almost abolished after the removal of the endothelium. The acetylcholine-induced relaxation was negligible both in unrubbed and rubbed helical strips. These functional data confirm the morphological analysis that the standard helical strips do not have an intact endothelium.

These results confirm that acetylcholine-induced relaxation is mediated by the release of EDRF (Furchgott & Zawadzki, 1980), which has been characterized as nitric oxide (NO) or a chemically related species (Palmer, Ferrige & Moncada, 1987; Myers, Guerra & Harrison, 1989; Myers, Minor, Guerra, Bates & Harrison, 1990; La, Li & Rand, 1996). It is well known that acetylcholine evokes endothelial NO biosynthesis and release via activation of muscarinic receptors (Ignarro, Harbison, Wood & Kadowitz, 1986).

The endothelium-dependent relaxations mediated by acetylcholine are not caused by factors such as prostacyclin (Moncada, Gryglewski, Bunting & Vane, 1976). Indeed, the present study (data not shown) provide evidence that indomethacin did not alter the depressor response to acetylcholine, suggesting a lack of involvement of prostanoids in the vasodilatory responses to this drug. These results are in accordance with the findings of Wang, Poon & Pang (1993a).

To better evaluate the functionality of the endothelium in the vascular preparations considered, the present authors tested the influence of L-NAME, an inhibitor of nitric oxide synthase (Moncada, Palmer & Higgs, 1991), on the basal tone, on noradrenaline-induced tone and on the relaxation induced by acetylcholine on tissues precontracted by noradrenaline.

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In vascular endothelium, the production of NO is subject to complex control. There is a basal production of NO which exerts a vasodilator action on endothelium-containing arterial rings (Martin, 1988). NO is stimulated by a large number of biological mediators such as acetylcholine (Furchgott & Zawadzki, 1980). Recent reports have suggested differences in the effects of drugs on basal and agonist-stimulated activity of NO (Randall & Griffith, 1991).

When L-NAME was tested on noradrenaline-induced contraction, it only slightly increased the contraction of the intact ring preparations in a statistically non- significant manner, while it had no effect on both the helical strips and the ring preparations without endothelium, even on the standard helical strips. These findings are in partial disagreement with those obtained in the rabbit isolated pulmonary artery by MacLean *et al.* (1993) and Laight, Matz, Caesar, Carrier & Anggard (1996); however, the present data obtained by using L-NNA show a strong potentiation of noradrenaline response on unrubbed ring preparation.

The slight potentiating influence of L-NAME on the noradrenaline response of unrubbed rings demonstrates that EDRF (NO) is probably one of the factors that can modulate the response of vascular tissue to noradrenaline. An additional hyperpolarizing factor, that is L-NAME resistant and different from EDRF (Taylor & Weston, 1988; Rand & Garland, 1992) may play a role in modulation of the noradrenaline response. Rubbed vessel preparations or standard helical strips, which morphological studies demonstrated to be devoid of a functional endothelium, lacked both releasing factors. In such preparations, the present author observed a statistically significant increase in muscle contraction in response to noradrenaline, as compared to that obtained in unrubbed rings in the presence or absence of L-NAME.

The influence of L-NAME on acetylcholine-relaxation was only studied in the endothelium-intact ring preparations. L-NAME (3×10^{-5} M, 1×10^{-4} M) reduced, but did not abolish, the acetylcholine-induced relaxation. Even a massive concentration of L-NAME (3×10^{-4} M) caused no further inhibition of the L-NAME-resistant relaxation. The partial inhibition by L-NAME of depressor responses to acetylcholine is in accordance with published studies (Rees, Palmer, Schlz, Hodson & Moncada, 1990; Wang, Poon & Pang, 1993b) and it has been suggested that this is caused by the partial involvement of NO biosynthesis in the vasodilatory response to acetylcholine (Moncada *et al.*, 1991). The relaxation response to acetylcholine that is resistant to the inhibition of EDRF synthesis is most likely to be caused by an EDRF-independent mechanism: in addition to prostacyclin (PGI_2) and NO, the vascular endothelial cells can release an as-yet unidentified hyperpolarizing factor in response to vasodilators, the endothelium-derived hyperpolarizing factor (EDHF) (Taylor & Weston, 1988; Hatake, Wakabayashi & Hishida, 1995; Drummond & Cocks, 1996).

In the light of the present results, it must be stressed that the acetylcholine-relaxation in ring preparations with intact endothelium in the presence of L-NAME is similar to that obtained in unrubbed and rubbed helical strip preparations in the absence of L-NAME; therefore, it can be concluded that the standard helical strip, prepared according to Furchgott & Badrakom (1953), is devoid of functional endothelium, a finding that was confirmed by morphological studies.

This conclusion can also be drawn from results obtained by Furchgott & Badrakom (1953) on aorta helical strips in which relaxation was never obtained with acetylcholine. Furchgott & Zawadzky (1980) demonstrated that the relaxation of isolated preparations of aorta by acetylcholine requires the presence of endothelial cells. This response was obtained using transverse rings or transverse strips, but not helical strips of aorta. The loss of the relaxing response to acetylcholine in aortic preparations (rings and transverse and helical strips) is caused by an unintentional rubbing of the intimal surface, which is usually the case during the preparation of helical strips (Furchgott, 1983).

Beckeringh *et al.* (1984) also stressed the absence of endothelium in the helically cut preparation in contrast to the ring preparation of the rat thoracic aorta. The present authors speculate that this is the reason that the efficacy and potency of α-adrenoceptor agonists are higher in the helically cut strips than in the ring preparations, as shown by comparison with the results obtained by Macia *et al.* (1984).

Alosachie & Godfraind (1986) investigated the role of the endothelium in modifying the contractile response to noradrenaline and the antagonistic action of prazosin. The experiments were carried out on intact rat aorta or endothelium-stripped rings. The present authors have come to the conclusion that the efficacy for noradrenaline was lower in the presence than in the absence of endothelium, and that prazosin acts as a non-competitive antagonist in the presence of endothelium and as a competitive antagonist in the absence of endothelium. The interference between endothelial factors and adrenergic transmission has recently been reviewed (Kaneco & Sunano, 1993; Rubanyi & Polokoff, 1994; Wright, Harling, Kendall & Wilson, 1995; Fujimoto & Itoh, 1995; Richer, Domergue, Vincent & Giudicelli, 1996; Pannanagpetch & Woodman, 1996).

In conclusion, even though all possible care was taken to make the standard helical strips, the endothelium was greatly damaged. It should be stressed that this conclusion was based on pharmacological, functional and morphological studies. A deterioration of the endothelial functionality brings an increase in the potency of noradrenaline and an increase in the noradrenaline maximum response of the helical strip preparations in comparison with the ring preparations with endothelium. These modifica-

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tions must be taken into consideration in those studies aimed at characterization of the adrenoceptors in isolated vascular tissues in which noradrenaline is the reference substance. Caution must be observed when comparing the potency of agonists on different vascular preparations and when results obtained from *in vitro* studies are extrapolated to the *in vivo* situation.

This conclusion, although drawn from a study of noradrenaline activity, might be generalized to other pharmacological substances that exhibit vascular activity modulated by endothelial derived factors.

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