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Interactions of nitric oxide synthase inhibitors and dexamethasone with α-adrenoceptor-mediated responses in rat aorta

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1 The effects of N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA), their D-isomers, and dexamethasone on noradrenaline (NA)-induced contractions and antagonism by α-adrenoceptor antagonists, have been investigated in rat isolated thoracic aortic rings with/without endothelium.

2 NA produced concentration-dependent contractions of isolated aortic rings with EC_{50} values of 2.41 ± 0.54 ($n = 21$) and 28.00 ± 8.50 ($n = 25$) nM for endothelium-denuded and -intact preparations respectively. Acetylcholine (ACh) relaxed NA-precontracted rings with intact, but not those denuded of endothelium.

3 Treatment with L-NAME (1–30 μM), or L-NMMA (10–500 μM), but not their D-isomers, resulted in an endothelium-dependent enhancement of NA-induced contractions. Pre-treatment, *in vitro*, with 0.5 μM dexamethasone neither directly potentiated, nor influenced L-NAME-induced potentiation of NA-mediated contractions in endothelium-intact rings; however, dexamethasone pretreatment reduced EC_{50} values for NA, and also prevented L-NAME-induced potentiation, in denuded rings equilibrated for 5 h under resting tension.

4 In both intact and denuded rings, phentolamine, prazosin and WB 4101 shifted NA concentration-response curves to the right; L-NAME, and also L-NMMA, but not their D-isomers, reversed the blockade as indicated by significant decreases in NA dose-ratios. In denuded rings, reversal by L-NAME or L-NMMA was prevented following pretreatment with dexamethasone.

5 Following treatment with 5 or 50 nM phenoxybenzamine (PBZ), NA concentration-response (C-R) curves were shifted to the right with marked depression of maximal responses; 100 μM L-NAME reversed the antagonism in both endothelium intact and denuded rings. However, 500 nM PBZ treatment resulted in complete abolition of the responses to NA, and contractions were not restored by either L-NAME or L-NMMA.

6 5-Hydroxytryptamine (5-HT)-induced contractions of aortic rings were potentiated by endothelium denudation and also by L-, but not D-, NAME. 5-HT-induced contractions were non-competitively antagonized by 10 nM ritanserin, and 100 μM L-NAME partially reversed the antagonism in intact but not denuded rings.

7 It is concluded that the inhibition of constitutive endothelial NO synthase and inducible smooth muscle NO synthase accounts for the ability of L-NAME, and L-NMMA, to potentiate the effects of agonists and reduce α-adrenoceptor antagonism in endothelium-intact and denuded rings. Furthermore, endothelial cell removal/damage triggers the induction of a smooth muscle NO synthase.

Keywords: Rat aorta; α-adrenoceptors; NO synthase inhibitors; inducible NO synthase; dexamethasone

Introduction

It is widely accepted that vascular endothelial cells (Palmer *et al.*, 1988; Palmer & Moncada, 1989; Ignarro, 1989), and a variety of other mammalian tissues and cell types such as cerebellum (Garthwaite *et al.*, 1988), stomach (Desai *et al.*, 1991), rat isolated aortae (Schini & Vanhoutte, 1991) and macrophages (Moncada *et al.*, 1989; Collier & Vallance, 1989), synthesize nitric oxide (NO) from L-arginine. Furthermore, it is also recognized that NO is the principal mediator of the actions of endothelium-derived relaxing factor (EDRF) on vascular smooth muscle (Palmer *et al.*, 1987). In rat isolated aortic rings, the presence of a functional endothelium has been shown to depress the contractile actions of α-adrenoceptor agonists (Allan *et al.*, 1983; Egleme *et al.*, 1984; Lues & Schumann, 1984; Carrier & White, 1985); an action attributed to the spontaneous release of EDRF and subsequent physiological antagonism of the contractile process. Thus endothelial denudation, and inhibitors of EDRF, such as methylene blue and oxyhaemoglobin, usually augment vascular contractions to a variety of agonists (Martin *et al.*, 1986; Miller *et al.*, 1988).

The role of the endothelium in the modulation of the binding of subtype-specific adrenoceptor radioligands to vascular α_1- and α_2-adrenoceptor sites was first described by Carman-Krzan (1985). It was reported that the removal of endothelium caused an increase in the density of specific α_1-adrenoceptor binding, with little effect on the affinity of the ligand for the receptor. Carman-Krzan (1985) concluded that the loss of endothelial cells may uncover otherwise undetectable receptor sites in the bovine thoracic aorta. Subsequently, Alosachie & Godfraind (1988) showed that the vascular endothelium modified prazosin-mediated antagonism of noradrenaline action: noncompetitive in the presence, and competitive in the absence of endothelium; a finding that was explained in terms of their earlier proposal (Alosachie & Godfraind, 1986) i.e. reduction in agonist efficacy and receptor reserve by EDRF. Perhaps more surprising was the demonstration by Mostaghim *et al.* (1988) that α-antagonists such as phentolamine, prazosin and yohimbine, caused endothelium-dependent relaxations of rat

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thoracic aortic rings; one interpretation of these data is that a component of what had been interpreted as adrenoceptor blockade may in fact be due to release of one or more bioactive substance(s) from the endothelium. If this interpretation is correct, the following hypothesis can be formulated: the degree of α-adrenoceptor antagonism produced by phentolamine, prazosin, yohimbine, or related compounds, should be less in endothelium-intact, compared to endothelium-denuded vascular tissue.

In the present study, we have tested this hypothesis, by examining the effects of endothelial denudation, and of the NO synthase inhibitors, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) on noradrenaline-induced contractions, and antagonism by a variety of α-adrenoceptor antagonists, in aortic rings with intact or denuded endothelium.

Methods

Male Sprague-Dawley rats (Charles River, PQ, Canada; 200–300 g) were stunned by a blow to the head and exsanguinated. Thoracic aortae were excised, cleaned of all connective tissue and cut into rings of approximately 3–4 mm length. Each ring was suspended between platinum hooks and mounted under 2 g passive tension in 10 ml organ baths containing physiological salt solution (PSS), maintained at 37°C and bubbled with 95% O_2/5% CO_2. Isometric tension was recorded with a force displacement transducer (Grass FT .03) coupled to a Grass polygraph model 7E. The PSS used had the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 12.5 and glucose 11.1. The pH of the solution after saturation with 95% O_2/5% CO_2 gas mixture was 7.4. As a routine, tissues were allowed to equilibrate for 1 h before the start of the experiments and where endothelial denudation was required, this was achieved mechanically by rubbing a piece of PE 90 tubing against the intimal surface of the aortic rings.

Experimental procedure

Protocol 1: This series of experiments was carried out to characterize the effects of L-NAME, L-NMMA, and their D-isomers on noradrenaline (NA) concentration-response (C-R) curves in aortic rings with/without endothelium. Control C-R curves were performed to NA and, upon reaching the maximal contractile response, 1 μM acetylcholine (ACh) was applied to ascertain the functional integrity of the endothelium; preparations which did not show >85% relaxation of the active tone were not included in our data. Thereafter, the tissues were washed and similar C-R curves performed 30 min after the addition of different concentrations of a NO synthase inhibitor.

Protocol 2: Three series of experiments were carried out under this protocol, all of which involved construction of C-R curves to NA before and after incubation with desired concentrations of: (a) α-adrenoceptor antagonists and, (b) α-adrenoceptor antagonists + NO synthase inhibitor L-NAME, L-NMMA, or their D-isomers, in aortic rings with/without endothelium. The first series examined the ability of the reversible antagonists, phentolamine, prazosin and WB 4101 to antagonize NA-induced contractions, and the influence of L-, and D-, NAME and NMMA on such effects. In these studies, tissues were equilibrated in PSS for 1 h, followed by control NA C-R curves (routinely performed twice to ensure consistency), and, then, incubation with each of the antagonists for 30 min before reestablishing C-R curves and calculations of dose-ratios for NA. Tissues were subsequently washed and incubated with a combination of the antagonist under study plus a desired concentration of either L-NAME and L-NMMA. The second series of experiments was designed to study the influence of the glucocorticoid, dexamethasone, on the interaction of L-NAME with NA-induced contractions, and the effects of prazosin thereon. The procedure was identical to that described in the first series above, except that experiments were performed in PSS with or without 0.5 μM dexamethasone, and tissues were equilibrated for either a 1- or a 5-h period. In the third series, the α-adrenoceptor alkylating agent phenoxybenzamine (PBZ) was used instead of the reversible antagonists. Thus, NA C-R curves were established, the aortic rings were then incubated with the alkylating agent for 30 min, washed in PBZ-free PSS for 2 h, and agonist responses alone, and in the presence of L-NAME (100 μM), reestablished.

Protocol 3: Experiments in this series examined the influence of L-, or D-NAME, on 5-hydroxytryptamine (5-HT) C-R curves and on the 5-HT/ritanserin interactions in aortic rings with/without endothelium. The procedure was as described for the first series of experiments in protocol 2 above.

Drugs

Noradrenaline hydrochloride, acetylcholine hydrobromide, N^G-nitro-L-arginine methyl ester, 5-hydroxytryptamine maleate, and dexamethasone were purchased from Sigma Chem. Co. (St. Louis, MO, U.S.A.); ritanserin, phenoxybenzamine hydrochloride and WB 4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane hydrochloride) were purchased from Research Biochemicals, Inc. (Natick, MA, U.S.A.); N^G-nitro-D-arginine methyl ester (Bachem Inc., Torrance, CA, U.S.A.); N^G-monomethyl-L-arginine (Chemical Dynamics Corp., Plainfield, NJ, U.S.A.). Phentolamine hydrochloride (Regitine) and prazosin hydrochloride were generously supplied by Ciba-Geigy Corp. (Summit, NJ, U.S.A.) and Pfizer Canada Inc. (Arnprior, Ont., Canada) respectively. N^G-monomethyl-D-arginine acetate hemihydrate was a gift from Dr S. Moncada, The Wellcome Research Laboratories, Kent, U.K.

Stock solutions of NA, 5-HT, and ritanserin were prepared in 0.1 M HCl; all other compounds dissolved freely in PSS except for prazosin which required sonication.

Data analysis

Results are presented as means ± s.e.mean of either the percentage maximal responses or absolute tension measurements. Differences between the mean values were determined by Student's *t* test for paired and unpaired observations, and were regarded as significant when $P<0.05$. NA dose-ratios, defined as the ratios of NA EC_{50} values in the presence to values in the absence of a competitive antagonist, were calculated from plots of individual experiments under the various applicable experimental conditions.

Results

Effects of endothelial denudation, L-NAME and L-NMMA on NA-induced contractions

NA (0.1–1000 nM) produced concentration-dependent contraction of rat isolated aortic ring segments with AC_{50} values (in nM) of 2.41 ± 0.54 ($n=21$) and 28.00 ± 8.50 ($n=25$) nM for endothelium-denuded and intact preparations respectively. Although the EC_{50} values were significantly ($P<0.05$) different, no differences in the E_{max} were apparent. Neither L-NAME nor L-NMMA, within the concentration range 1–30 μM and 10–500 μM respectively, affected basal aortic tone, however, the L- but not the D-isomers of these inhibitors caused a leftward shift of NA concentration-response curves with marked increases in the maximal response in endothelium-intact aortic rings (Figure 1). No enhancement was observed in endothelium-denuded rings.

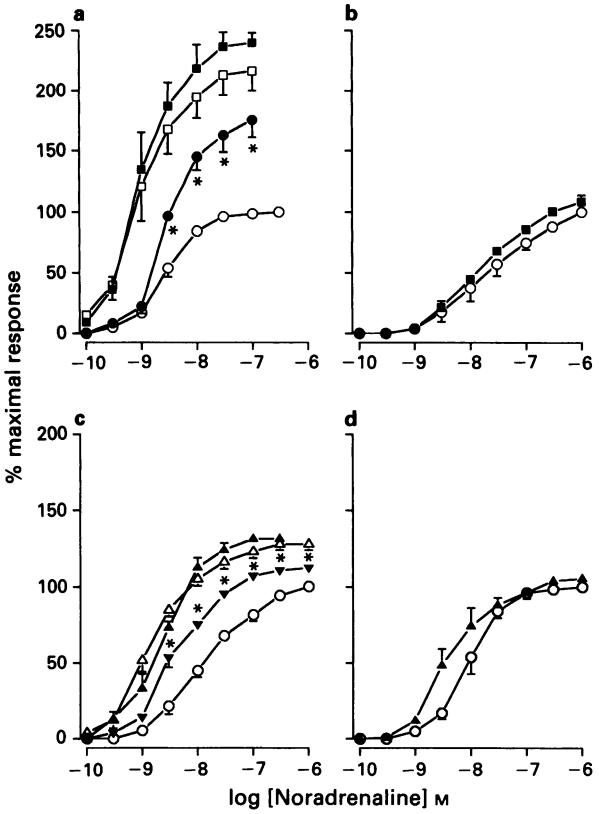

Figure 1 The effects of N^G-nitro-L-arginine methyl ester (L-NAME) (a) N^G-monomethyl-L-arginine (L-NMMA) (c) and their corresponding D-isomers (b,d) on concentration-response curves to noradrenaline (NA) in rat aortic rings with an intact endothelium. In all panels, (○) represent control responses, while (●), (□) and (■) respectively represent the responses in the presence of 1, 10 and 30 μM L-, or D-NAME in (a) and (b); and, (▼), (△) and (▲) respectively represent the responses in the presence of 10, 100 and 500 μM L-, or D-NMMA in (c) and (d). Each point on the graph represents the mean ± s.e. ($n = 5-12$). *Denotes statistically significant difference ($P < 0.05$) from corresponding control values.

NA antagonism by phentolamine, prazosin and WB 4101: influence of NO synthase inhibitors

Regardless of whether the endothelium was intact or denuded, the concentration-response curves to NA were shifted to the right by 0.3 μM phentolamine, 3.0 nM prazosin and 5 nM WB 4101 without a change in the maximal responses. L- but not D-NAME, at either 10 or 100 μM caused significant decreases in the ability (measured at dose-ratios) of prazosin (Table 1), and also phentolamine and WB 4101 (Figure 2), to inhibit NA-induced contractions. NA concentration-response curves, in the presence of the antagonist and L-NAME, were shifted leftwards, towards the control curve, and this effect occurred both in endothelium-intact and denuded rings, L-NMMA, at 100 or 500 μM, also reduced the dose-ratios of phentolamine and prazosin on NA C-R curves (Figure 3).

Effects of dexamethasone

Treatment with 0.5 μM dexamethasone did not significantly alter the EC_{50} values for NA in either endothelium-intact or denuded rings, equilibrated in PSS for 1 h. For example, NA EC_{50} values (in nM) in the absence, and presence, of dexamethasone pretreatment were: 23.8 ± 7.0 ($n = 31$) versus

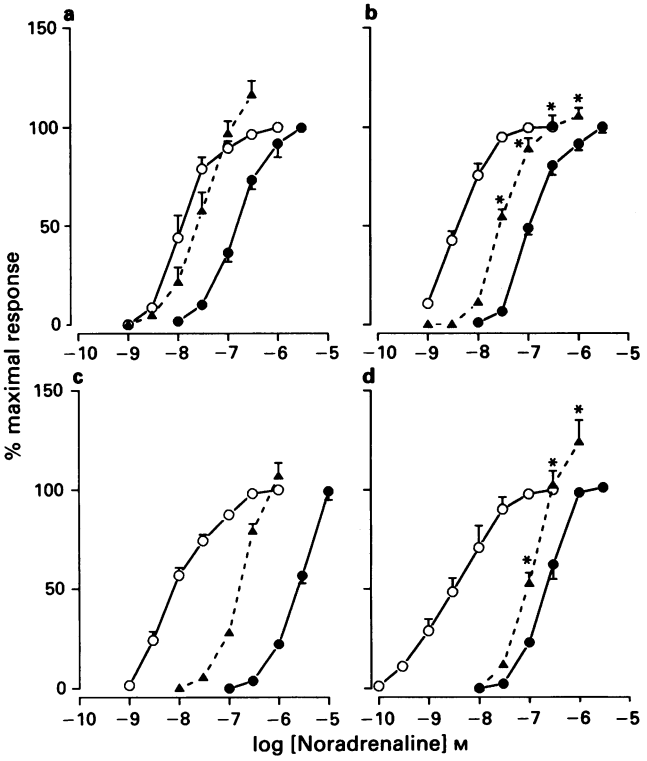

Figure 2 The effects of N^G-nitro-L-arginine methyl ester (L-NAME) on the antagonism of concentration-response curves to noradrenaline (NA) by 0.3 μM phentolamine (a,b) and 5 nM WB 4101 (c,d) in rat isolated aortic rings with intact (a,c) or denuded (b,d) endothelium. In all panels, (○), represent NA control responses, while (●) and (▲) respectively represent the responses to NA in the presence of the antagonists alone and antagonist + 100 μM L-NAME. Each data point on the graphs represents the mean ± s.e. ($n = 8$). *Denote statistically significant difference ($P < 0.05$) from the corresponding responses in the presence of the antagonist alone.

Table 1 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) and D-NAME, and prazosin, on noradrenaline (NA)-mediated responses (expressed as dose-ratios) in rat isolated aortic rings with/without endothelium and in the absence/presence of 0.5 μM dexamethasone (Dex)

	Prazosin (3 nM)	+ *L-NAME* (10 μM)	+ *L-NAME* (100 μM)	+ *D-NAME* (100 μM)
+ Endo	95.60 ± 14.77 ($n = 12$)	31.05 ± 5.06* ($n = 8$)	7.97 ± 2.77* ($n = 8$)	87.75 ± 13.46 ($n = 5$)
+ Endo, + Dex	117.68 ± 30.88 ($n = 6$)	12.99 ± 4.74* ($n = 6$)	4.05 ± 2.07* ($n = 6$)	
(−) Endo	105.35 ± 20.65 ($n = 11$)	52.49 ± 20.08* ($n = 8$)	19.89 ± 8.01* ($n = 8$)	112.96 ± 34.30 ($n = 5$)
(−) Endo, + Dex	106.78 ± 15.47 ($n = 6$)	120.65 ± 31.74 ($n = 6$)	114.19 ± 33.04 ($n = 6$)	

*Denote significant ($P < 0.05$) differences from corresponding controls.

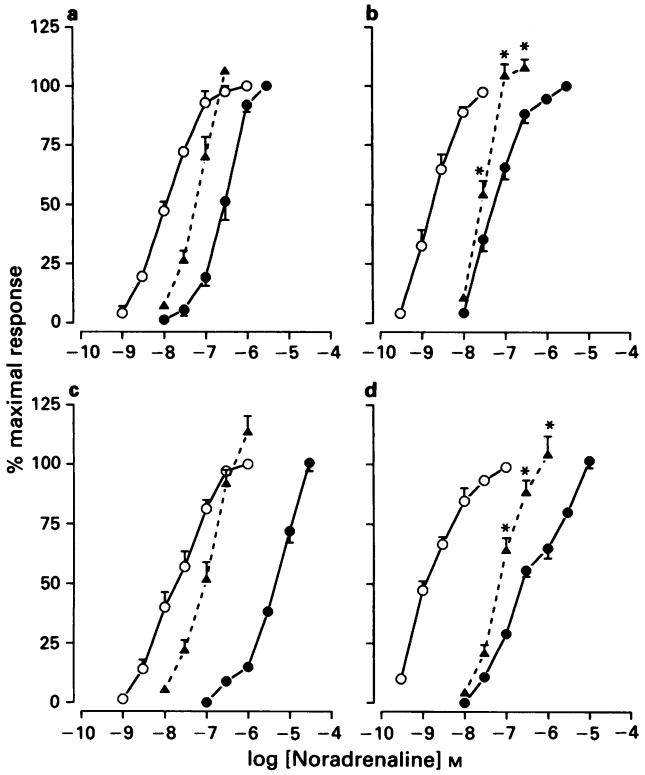

Figure 3 The effects of N^G-monomethyl-L-arginine (L-NMMA) on the antagonism of concentration-response curves to noradrenaline (NA) by 0.3 μM phentolamine (a,b) and 3 nM prazosin (c,d) in aortic rings with intact (a,c) or denuded (b,d) endothelium. In all panels, (○), represent NA control responses, while (●) and (▲) respectively represent the responses to NA in the presence of the antagonist alone, and antagonist + 500 μM L-NMMA. Each data point on the graphs represents the mean ± s.e. ($n = 5$). *Denote statistically significant difference from the corresponding responses in the presence of the antagonist alone.

16.1 ± 4.6 ($n = 6$) respectively in endothelium-intact rings; and, 2.4 ± 0.4 ($n = 21$) versus (1.9 ± 0.5 ($n = 11$) respectively in endothelium-denuded rings. However, following 5 h of tissue pre-equilibration in PSS, EC_{50}s for NA were significantly ($P < 0.05$) higher than comparative values obtained during shorter-term pre-equilibration (Table 2). EC_{50}s for NA in denuded, but not intact rings, pre-equilibrated for 5 h with PSS containing 0.5 μM dexamethasone were significantly lower. Treatment with L-NAME (100 μM) potentiated NA-induced contractions in 5 h PSS pre-equilibrated, endothelium-intact and denuded rings; however, there was no such potentiation in denuded rings equilibrated in PSS for only 1 h. The presence of dexamethasone in the PSS selectively antagonized L-NAME-induced potentiation in denuded rings (Figure 4). Regardless of whether the endothelium was intact or denuded, NA dose-ratios, with prazosin as the antagonist, were similar in control versus dexamethasone-treated rings (see Table 1); L-NAME at 100 μM, still significantly reduced the dose-ratios in dexamethasone-treated rings with intact endothelium, but, it had no significant effect in endothelium-denuded rings (see Table 1).

Table 2 EC_{50} values (nM) for noradrenaline (NA) in endothelium-intact or denuded aortic rings, and pre-equilibrated for 1 or 5 h, in physiological salt solution with/without 0.5 μM dexamethasone (Dex)

	− *Dex*		+ *Dex*	
	1 h	5 h	1 h	5 h
+ Endo	23.8 ± 7.0 ($n = 31$)	114.0 ± 19.1[a] ($n = 5$)	16.1 ± 4.6 ($n = 6$)	82.3 ± 19.2[a] ($n = 4$)
− Endo	2.4 ± 0.4 ($n = 21$)	33.6 ± 7.8[a] ($n = 6$)	1.9 ± 0.5 ($n = 11$)	14.4 ± 3.3[a,b] ($n = 5$)

Data are expressed as means ± s.e., (n).
[a] and [b] respectively denote statistically significant differences ($P < 0.05$) from corresponding values obtained after 1 h pre-equilibration, and in the absence of dexamethasone.

Interaction of L-NAME with NA after treatment with PBZ

In aortic rings with/without endothelium, and treated with 5 or 10 nM PBZ, to alkylate α_1-adrenoceptors, contractions initiated by NA had a reduced E_{max}. Pretreatment with L-NAME (100 μM) restored responsiveness to NA in rings with/without endothelium (Figure 5). However, following treatment with 0.5 μM PBZ, both endothelium intact and denuded rings failed to respond to NA; L-NAME, in concentrations up to 1 mM, failed to reverse this inhibition (see Figure 5).

Are the effects of L-NAME specific for α-adrenoceptor-mediated events?

We also examined the effects of L-NAME on 5-HT-mediated contractions in the absence and presence of the 5-HT_2 antagonist, ritanserin, in both endothelium-intact and denuded aortic rings. 5-HT (0.1–30 μM) initiated concentration-related contractions of rat aortic rings with E_{max} of 0.64 ± 0.09 and 1.46 ± 0.18 g tension respectively for endothelium-intact and denuded rings. L-NAME (100 μM) potentiated the 5-HT contractions in intact but not in denuded

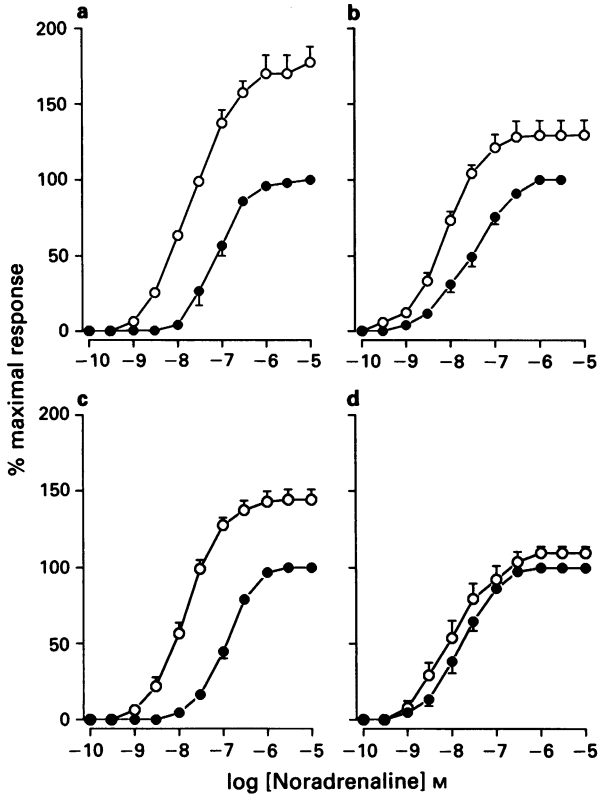

Figure 4 Potentiating effect of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) on concentration-response curves to noradrenaline (NA) in endothelium-intact (a,c) and denuded (b,d) aortic rings after 5 h equilibration in normal PSS (a,b) or PSS containing 0.5 μM dexamethasone (c,d). In both panels, (●) and (○) respectively represent the responses to NA in the absence and presence of 100 μM L-NAME. Each data point is mean ± s.e. ($n = 5$).

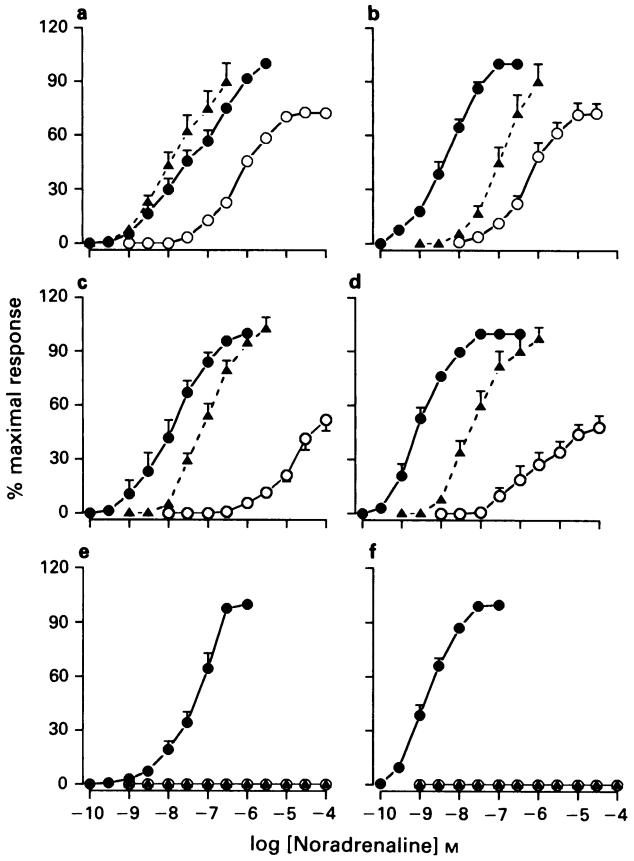

Figure 5 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) after antagonism of contractions to noradrenaline (NA) by 5 (a,b), 50 (c,d) and 500 (e,f) nM phenoxybenzamine (PBZ) in endothelium-intact (a,c,e), and denuded (b,d,f) aortic rings. In all panels, (●) represent control responses to NA, while (○) indicate NA responses after treatment with the antagonist alone, and (▲), antagonist treatment + 100 μM L-NAME. Each data point represents the mean ± s.e. ($n = 5$).

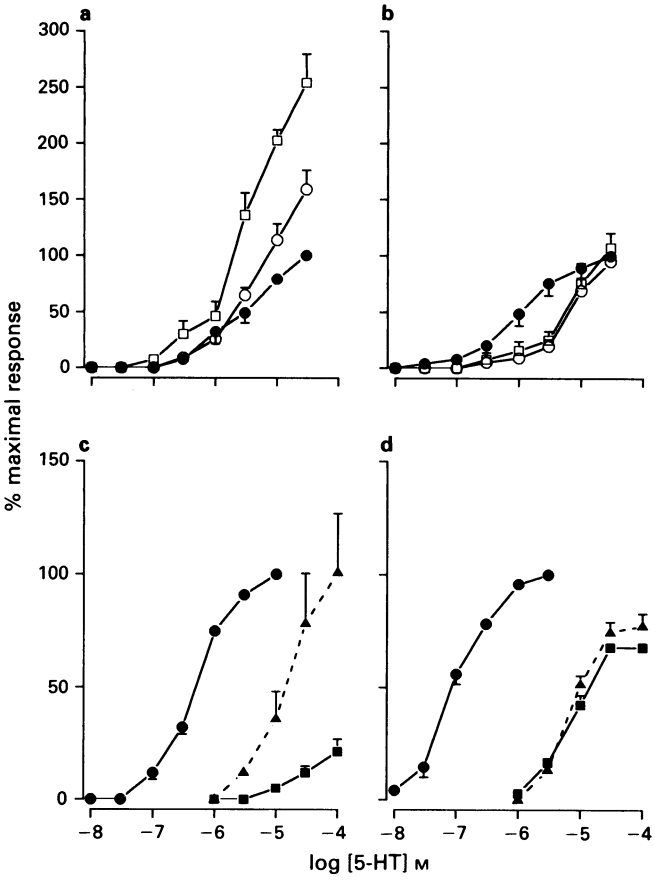

Figure 6 Effect of N^G-nitro-L-arginine methyl ester (L-NAME) (a) and D-NAME (b) on the concentration-response curves to 5-hydroxytryptamine (5-HT), and, of L-NAME on the antagonism of 5-HT responses by ritanserin (c,d) in aortic rings with (c) or without (d) endothelium. For (a,b), (●), represents the control responses, while (○) and (□) respectively represent responses in the presence of 10 and 100 μM L-, or D-NAME. In (c,d), (●), represents the control 5-HT responses, while (■) and (▲) respectively represent 5-HT responses in the presence of 10 nM ritanserin alone, and ritanserin + 100 μM L-NAME. Each data point represents the mean ± s.e. ($n = 4$).

rings (Figure 6a). Ritanserin, 10 nM, non-competitively antagonized responses to 5-HT, and treatment with 100 μM L-NAME resulted in a partial restoration of 5-HT contractions in endothelium-intact, but not in denuded rings (Figure 6b).

Discussion

The ability of endothelium-derived nitric oxide (EDNO) to mediate vascular relaxation (Moncada *et al.*, 1987) and to modulate agonist-induced contractile responses of the vasculature (Miller *et al.*, 1988) is very well documented. The data from the present study, are in accord with these established facts. However, this study has also provided evidence that constitutive, as well as inducible, NO synthase(s) contribute to the actions of L-NAME and L-NMME, viz enhancement of NA contractions, and reduction of the effects of α-adrenoceptor antagonists in endothelium-denuded rings. Evidence that a smooth muscle NO synthase contributed to the effects of NO synthase inhibition was provided by the ability of dexamethasone to inhibit the enhancement of responses to NA following either L-NAME or L-NMMA treatment, and to reverse antagonism of NA by α-adrenoceptor antagonists in denuded rings.

The role of the rat aortic endothelium as a generator of NO, and modulator of contractile response, is clearly evident in the present study: the NO synthase inhibitors produced an endothelium-dependent enhancement of NA-, and also 5-HT-, induced contractions, with L-NAME, being more potent than L-NMMA. As expected, the D-isomers of both of these NO synthase inhibitors were ineffective. It has previously been demonstrated that L-arginine, but not its D-enantiomer, is the substrate for NO synthesis (Palmer *et al.*, 1988) and that L-NMMA, but not its D-isomer (Rees *et al.*, 1989) and L-NAME are competitive inhibitors of the endothelial NO synthase (Rees *et al.*, 1990b); the latter having been shown to be an irreversible inhibitor of the brain NO synthase (Dwyer *et al.*, 1991).

A second aspect of our study relates to the interaction between NO synthase inhibitors and α-adrenoceptor antagonists in aortic rings with/without endothelium. The dose-ratios for NA in the presence of the competitive α-adrenoceptor antagonists, phentolamine, prazosin and WB 4101 were significantly reduced by L-NAME and L-NMMA in rings with intact or denuded of endothelium. It might be argued that the reduction of NA dose-ratios in endothelium-intact rings results from the inhibition of endothelial NO production by L-NAME, or L-NMMA. However, this does not explain the stereospecific reversal of α-adrenoceptor blockade that occurred in denuded rings. The latter data suggest the involvement of an inducible smooth muscle NO synthase in denuded rings. The ability of L-NAME/L-NMMA to reverse α-adrenoceptor blockade may thus relate to the approximately 5 h incubation period in PSS following the initial equilibration of the aortic rings and the completion of the

experiment (see protocol 2). Evidence implicating the inducible vascular smooth muscle (VSM) NO synthase in the reversal phenomenon include: (a) the significant increases in NA EC_{50} values for tissues equilibrated in PSS for 5 h versus 1 h; treatment with L-NAME restored the EC_{50}s to near control values. (b) Dexamethasone prevented the effects of L-NAME on NA C-R curves and also on the reversal phenomenon in denuded rings. The induction of smooth muscle NO synthase, which can be prevented by the glucocorticoids (Rees *et al.*, 1990a), has previously been demonstrated to require a lag period of 2 h, with maximal inducible enzyme activity detectable in 6–12 h (Radomski *et al.*, 1990). Furthermore, Schini & Vanhoutte (1991) have also shown that rat thoracic aorta is endowed with an inducible NO synthase which can be detected after prolonged (8 h) equilibration in PSS.

The ineffectiveness of dexamethasone on both potentiation and reversal phenomenon in endothelium-intact rings is surprising in view of the findings by Radomski *et al.* (1990) that the vascular endothelium also expresses an inducible, Ca^{2+}-independent NO synthase after activation *in vitro* with lipopolysaccharide (LPS) and interferon-γ (IFN-γ). In our study, no such activation of the inducible NO synthase was apparent, except in denuded rings, thus suggesting that endothelial denudation, or damage, may, in addition to LPS and IFN-γ, serve as a trigger for the induction of the smooth muscle NO synthase and also that this enzyme system may function as a physiological safeguard when there is a dysfunction in the constitutive synthase system.

It has been known for some time that glucocorticoids can potentiate the responses of vascular smooth muscle to the effects of catecholamines (Kalsner, 1969; Yard & Kadowitz, 1972). Glucocorticoid receptors, which bind dexamethasone, have been demonstrated in VSM cells (Dural *et al.*, 1977; Kornel *et al.*, 1981) thus suggesting a direct effect of glucocorticoids on VSMs as the basis, at least in part, for the pathogenesis of glucocorticoid-induced hypertension (Romas-Frendo *et al.*, 1985). Our data also indicate a significant reduction of the EC_{50} value for NA in the presence of dexamethasone in endothelium-denuded aortic rings, but not in the endothelium-intact rings where active synthesis of NO mediated by a constitutive enzyme population is presumably uninterrupted. Thus, considering that the period of time following dissection was comparable for both endothelium-denuded and intact tissues, it is compelling to conclude that the effects of dexamethasone are mediated, in denuded tissues, by an inhibition of the production of an inducible aortic smooth muscle NO synthase rather than a direct receptor effect.

The contractile effect of NA on the rat thoracic aorta is mediated by the activation of α_1-adrenoceptors, possibly coupled to two different signal transduction processes, one of which mobilizes intracellular Ca^{2+} and the other activates the translocation of extracellular Ca^{2+} (Mir & Fozard, 1989; Oriowo & Ruffolo, 1992; Oriowo *et al.*, 1992). Our study of the effect(s) of L-NAME in PBZ-treated tissues was designed to determine the dependency, or otherwise, of NO synthase activation on the α_1-adrenoceptor-mediated contractile process. In aortic rings with/without endothelium, contractions occurred to NA, and L-NAME evoked a reversal of the inhibition of NA contractile responses in aortic rings with/without endothelium. L-NAME reversed the antagonism of NA contractions induced by PBZ treatment, and, when PBZ concentration was sufficiently high to cause total abolition of NA-induced contractions, treatment with L-NAME failed to reverse the inhibition in rings with/without endothelium. It thus seems that the reversal of α-adrenoceptor blockade by L-NAME is related to the ability of the agonist to evoke aortic contractions. It is unclear, however, why L-NAME should fail to reverse the antagonism of 5-HT_2 receptors by ritanserin, in endothelium-denuded rings. Our expectation was that within the time frame of these experiments, 4–5 h, vascular NO synthase activity should have been induced.

In conclusion, the current data has produced evidence in support of two types of NO synthase activities in rat isolated aortic rings: (a) endothelial NO synthase, the action of which modulates contractile responses to NA and 5-HT; and (b) an inducible smooth muscle NO synthase(s), the induction of which is enhanced by endothelial cell removal and can be prevented by preequilibration with dexamethasone. Both enzymes are inhibited in a stereospecific manner by either L-, NAME or NMMA, with resultant increased response and sensitivity to vasoconstrictor influences.

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