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Effect of N^G-nitro-L-arginine methylester (L-NAME) on functional and biochemical α_1-adrenoceptor-mediated responses in rat blood vessels

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1 The modulation by N^G-nitro-L-arginine methylester (L-NAME) of α_1-adrenoceptor-mediated contraction was investigated on isolated segments of rat tail artery and aorta. The influence of L-NAME on inositol phosphates accumulation by α_1-adrenoceptor agonists was also investigated to elucidate the intracellular mechanisms responsible for this modulation.

2 In aorta but not in tail artery L-NAME (30 μM) enhanced the sensitivity (3.3 times) and the maximum contraction (E_{max}) induced by the full agonist, phenylephrine.

3 St-587, a partial α_1-adrenoceptor agonist, behaved as a weak agonist in the aorta (22.2% of phenylephrine E_{max}). However, when the same agonist was studied in tail artery rings a maximum contraction that was 78.4% of the phenylephrine induced E_{max} was reached.

4 L-NAME increased (3.3 times) the E_{max} for St-587 contraction in the aorta but not in the tail artery. Sensitivity to St-587 was slightly but significantly ($P<0.001$) enhanced (1.9 times) by L-NAME in tail artery segments.

5 Contractile responses to phenylephrine after partial alkylation with phenoxybenzamine were analyzed by the nested hyperbolic null method. To elicit 50% of E_{max} for contraction only 1.1% of the receptors in the tail artery and 21% of the receptors in the aorta need to be occupied. These results indicate a higher receptor reserve for the tail artery than the aorta.

6 In the tail artery but not in the aorta, St-587 activates phosphoinositide turnover. The presence of L-NAME was without effect on inositol phosphates accumulation induced by this partial α_1-adrenoceptor agonist.

7 The maximum contraction induced by phenylephrine, after partial α-adrenoceptor alkylation, was enhanced by L-NAME in tail artery rings. However, the NO synthase inhibitor was unable to modify the phenylephrine-induced accumulation of inositol phosphates in the presence of phenoxybenzamine.

8 These results indicate that the differences in St-587-induced contraction and the modulation by L-NAME of α_1-adrenoceptor-mediated contraction observed between the tail artery and aorta are associated with differences in receptor reserve. In addition, our biochemical studies indicate that the potentiating effect of L-NAME is independent of intracellular calcium release *via* phosphatidylinositol turnover.

Keywords: Rat aorta; tail artery; α_1-adrenoceptors; N^G-nitro-L-arginine methylester; phophoinositide hydrolysis; St-587

Introduction

The release from endothelial cells of an endothelium-derived relaxing factor (EDRF, Furchgott & Zawadzki, 1980) now identified as nitric oxide (NO, Palmer *et al.*, 1987) modulates contractile responses in blood vessels to several agonists (Allan *et al.*, 1983; Cocks & Angus, 1983; Godfraind *et al.*, 1985; Trezise *et al.*, 1992) among them α_1-adrenoceptor agonists. However, wide differences in the influence of endothelium on noradrenaline responses have been observed in several blood vessels (Malta *et al.*, 1986; Martin *et al.*, 1986; Alosachie & Godfraind, 1986). We have previously reported that removal of the endothelium increases sensitivity to noradrenaline in the aorta but not in the tail artery from Sprague-Dawley rats (Tabernero & Vila, 1995). In addition to NO, the endothelium releases other relaxing and contractile factors (Sanchez-Ferrer & Marin, 1990) that could play a role in the modulation of α_1-adrenoceptor-mediated responses. It has been reported that in the rat tail artery, sympathetic vasoconstriction is modulated by three endothelial factors (Thorin & Atkinson, 1994). Furthermore, responses mediated through partial α_1-adrenoceptor agonists seem to be affected to a greater extent than those induced by a full agonist (Godfraind *et al.*, 1985; Topouzis *et al.*, 1991).

To avoid the influence of endothelium-released factors other than NO on the α_1-adrenoceptor mediated responses, we first studied whether N^G-nitro-L-arginine methylester (L-NAME), (an arginine analogue that inhibits the production of NO from L-arginine), influenced in the same way as removal of the endothelium, the contractile responses induced by a full agonist (phenylephrine) and by a partial α_1-adrenoceptor agonist (St-587, De Jonge *et al.*, 1981) in the rat tail artery and aorta. To test the hypothesis that the differences in the influence of the endothelium between tail artery and aorta were due to differences in receptor reserve, responses to phenylephrine, a selective α_1-adrenoceptor agonist (Starke *et al.*, 1975), before and after partial alkylation with phenoxybenzamine were studied.

In relation to the biochemical mechanism underlying this modulation process it has been reported that guanosine 3': 5'-cyclic monophosphate (cyclic GMP) attenuates the accumulation of inositol phosphates induced by stimulation of α_1-

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adrenoceptors in rat aorta (Hirata *et al.*, 1990; Rapoport, 1986). Thus, we have also examined whether differences obtained with L-NAME on contractile responses mediated by α_1-adrenoceptors were also related to changes in the phosphoinositides turnover.

Methods

The experiments were performed on 3–4 month old male Sprague-Dawley rats (322 ± 14.8 g). The animals were killed by a sharp blow to the head and the thoracic aorta and tail artery quickly removed, cleaned of adherent tissue and placed in gassed (95% O_2, 5% CO_2) physiological salt solution (PSS) of the following composition (in mM): NaCl 112.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.1. Yohimbine (0.1 μM) was present throughout the experiment to prevent stimulation of α_2-adrenoceptors.

Contractile studies

Rings of thoracic aorta (5 mm) and proximal tail artery (2-3 mm) were set up in 20 ml organ baths containing PSS maintained at 37 ± 0.5°C and continuously gassed with 95% O_2, 5% CO_2. A resting tension of 19.6 mN (aorta) or 7.4 mN (tail artery) was applied and changes in tension recorded with a PIODEN (UF-1) isometric transducer attached to a Omniscribe pen recorder. The preparations were left to equilibrate for 1 h (aorta) or 30 min (tail artery) and tension was readjusted if necessary. The tissues were contracted 3 or 4 times with KCl 50 mM (aorta) or 75 mM (tail artery) every 5 min until the amplitude of contractile response was similar in magnitude. After a 20 min (tail artery) or 30 min (aorta) equilibration period each ring was contracted with noradrenaline (aorta: 0.01 μM; tail artery: 0.03 μM) and relaxed with acetylcholine (1 μM) to verify the functional state of the endothelium. Only preparations that relaxed by more than 60% were used. Rings were washed with PSS and after a further 30 min equilibration, a cumulative agonist concentration-effect (E/[A]) curve to phenylephrine (0.001–100 μM) or St-587 (0.01–100 μM) was constructed. The bath was then washed repeatedly with physiological salt solution until preparations reached their initial tension. Then, arterial rings were incubated with L-NAME (30 μM) or PSS for 30 min before construction of a second cumulative E/[A] curve to the agonist.

In the partial receptor inactivation exposure, 30 min after the initial cumulative E/[A] curve to phenylephrine, tissues were exposed for 10 min to the alkylating agent, phenoxybenzamine, at the selected concentrations (0.03 μM and 0.1 μM for tail artery, and 0.01 μM for aorta). The rings were then washed successively every 5 min for 30 min, after which the agonist concentration-response curve was repeated. Control experiments were run in parallel to check the reproducibility over time between two or three curves performed under the above mentioned conditions.

When the effects of L-NAME were studied on contractions elicited by phenylephrine after alkylation of the α-adrenoceptors by phenoxybenzamine, three E/[A] curves with agonist were elicited in the same tail artery ring. The protocol of partial inactivation with phenoxybenzamine (0.1 μM) described above was followed but after the second E/[A] curve with the alkylating agent, a third curve with phenylephrine with or without L-NAME (0.3 μM, 30 min incubation) was constructed.

Inositol phosphates assay

The thoracic aorta and tail artery were rinsed with buffered Krebs solution (KRB, composition in mM: NaCl 118.3, KCl 4.7, $CaCl_2$ 1.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1) equilibrated with 95%O_2, 5% CO_2. Three or five rats were used in each individual experiment to obtain sufficient material. The arteries were cleaned of adherent tissue and then cut into rings (1 mm for aorta, 4 mm for tail artery) and pooled. The rings were incubated at 37°C and aerated with 95%O_2, 5%CO_2 for 30 min in KRB. The Krebs solution was changed every 10 min. Subsequently, the vessels were labelled with 1 μM [^{3}H]-myoinositol (specific activity 18.8 Ci mmol^{-1}) in KRB buffer pH 7.4 equilibrated with 95%O_2, 5%CO_2 at 37°C for 2 h. After incubation, the rings were washed twice with KRB and a third time with KRB containing LiCl (10 mM). Two pieces of tail artery or three rings of aorta were incubated for 30 min in a final volume of 550 μl of KRB buffer containing LiCl 10 mM with or without L-NAME (30 μM). Samples were then stimulated with increasing concentrations of St-587 (0.1–100 μM) for 30 min under an atmosphere of 95%O_2, 5%CO_2. The reaction was stopped by the addition of 2 ml $CH_3OH/CHCl_3/HCl$ (40:20:1 V/V/V) mixture and the sample sonicated for 45 min at 2–3°C. After the addition of 0.63 ml $CHCl_3$ and 1.26 ml distilled water, the samples were centrifuged at 2500 r.p.m. for 10 min to facilitate phase separation. The aqueous phase was neutralized and applied to columns containing Dowex AG 1X8 formate form ion exchange resin previously equilibrated with 30 ml 10 mM Tris-formate, pH = 7.4. The columns were then washed with 15 ml of unlabelled *myo*inositol (5 mM) and 10 ml of 60 mM sodium formate/5 mM sodium borate. Total [^{3}H]-inositol phosphates were eluted by washing with 0.1 M formic acid in 1M ammonium formate according to the method of Berridge *et al.* (1982) and counted for radioactivity. The lipid layer remaining after removal of the aqueous phase was used for measurement of the [^{3}H]-phosphatidylinositols. Aliquots of the lipid phase (200 μl) were removed, left to evaporate overnight, and counted for radioactivity.

In a set of experiments a protocol of partial receptor inactivation with phenoxybenzamine similar to the one used for contractile studies was followed. The assay was run as described above but before the 2 h incubation period with [^{3}H]-*myo*inositol the rings were incubated for a further 10 min with phenoxybenzamine (0.1 μM) or KRB (control) and washed every 5 min for 30 min. After washing the vessels twice with KRB and a third time with KRB containing LiCl, the vessels treated with phenoxybenzamine were divided in two sets. From this point, the protocol was similar to the one described above except that three E/[A] curves were run in parallel: (a) control, (b) with phenoxybenzamine and (c) with phenoxybenzamine plus L-NAME.

Data analysis

Pragmatic logistic curve fitting: Each individual set of E/[A] curve data was fitted to a logistic function of the form:

$$E = \frac{\alpha[A]^m}{[EC_{50}]^m + [A]^m} \quad (1)$$

in which E and [A] are the pharmacological effect and the concentration of agonist, respectively; α, EC_{50} and m are the asymptote, location and slope parameters, respectively. Location parameters were actually estimated as negative logarithms (pEC_{50}, i.e., the concentration required to cause 50% of the maximum response).

Experimental points and results from pragmatic logistic curve fitting were expressed as mean ± s.e.mean, *n* is the number of rings for contractile studies and the number of experiments performed in triplicate in the inositol phosphates assay; it is indicated in the legends of the figures. Contractile responses were expressed as a percentage of the E_{max} (maximum contraction, expressed in mN) of the control curve.

Accumulation of [^{3}H]-inositol phosphates was calculated as the percentage of ^{3}H-inositol labelled lipids in each individual sample to correct for interexperimental variations in labelling and sample size. The results are expressed as a percentage of E_{max}. The statistical significance of the observed differences was assessed by Student's two-tailed *t* test for paired or un-

paired (contractile responses of phenylephrine *vs* St-587) observations. In all cases significance was set at a P value of less than 0.05.

Nested hyperbolic null method Data obtained from receptor inactivation experiments were analyzed by the nested hyperbolic null method (James *et al.*, 1989). This method, which is analytically simpler than the classical method of Furchgott (1966), involves fitting the control E/[A] curve data to equation 1 whilst simultaneously fitting the postinactivation E/[A] curve data to the following equation:

$$E = \frac{\alpha}{\left(\frac{EC_{50}}{qK_A[A']}(K_A + [A'](1-q))\right)^m + 1} \quad (2)$$

where q represents the fractional receptor concentration which remains following inactivation.

Experimental data were directly fitted to the mathematical models described above with the 'AR' programme (derivative-free, nonlinear, regression analysis) within BMDP statistical software package (Dixon, 1990) implemented on a Vax 6610 computer. It is assumed that estimates of K_A, EC_{50} are log-normally distributed; therefore, each of them is expressed as a logarithmic value.

Drugs and isotopes

(−)-Noradrenaline bitartrate, acetylcholine HCl, N^G-nitro-L-arginine methyl ester, lithium chloride, *myo*inositol and (−)-L-phenylephrine hydrochloride were purchased from Sigma Chemical Co; [^{3}H]-*myo*inositol from Amersham International; 2-(2-chloro-5-trifluormethyl-phenylimino)-imidazoline (St-587) from Boerhinger Ingelheim; phenoxybenzamine from Research Biochemical Incorporated (RBI). All drugs were prepared in physiological salt solution except noradrenaline which was prepared in 23 μM Na_2 EDTA. All other chemicals used were of analytical grade.

Results

The influence of L-NAME on E/[A] curves of phenylephrine and St-587 in both tail artery and aorta are shown in Figures 1 and 2, respectively. Phenylephrine behaved as a full agonist in both blood vessels giving an E_{max} similar to maximum noradrenaline contraction (results not presented). In tail artery (Figure 1a), L-NAME 30 μM neither significantly affected maximum contractions to phenylephrine (control: 26.9±4.5 mN; L-NAME: 27.5±5.7 mN, $n=6$) nor did it modify the pEC_{50} values (control: 5.9±0.08; L-NAME: 5.8±0.01, $n=6$). However, in aorta, the phenylephrine E/[A] curve was shifted to the left by L-NAME 30 μM (Figure 1b). The NO-synthase inhibitor increased not only the sensitivity (control: 7.3±0.04; L-NAME: 7.8±0.06, $n=6$, $P<0.0001$) but also the maximum contraction to phenylephrine (control: 25.5±2.06 mN; L-NAME: 32.2±2.7 mN, $n=6$, $P<0.005$).

Since vasoconstriction induced by partial agonists seem to be affected by the presence of NO to a greater extent than responses to full agonists (Godfraind *et al.*, 1985; Topouzis *et al.*, 1991) the effect of L-NAME on contractile responses induced by a partial α_1-adrenoceptor agonist such as St-587 was studied. This compound contracted the tail artery in a concentration-related manner (Figure 2a). Maximum contraction was slightly but significantly smaller than maximum contraction induced by phenylephrine (St-587: 21.1±1.5 mN, $n=6$; phenylephrine: 26.9±4.5 mN, $n=6$; $P<0.05$). In rat aorta (Figure 2b) St-587 behaved as a weak agonist on α_1-adrenoceptors showing a maximum contractile effect of 5.7±1.5 mN ($n=7$). In the tail artery, L-NAME slightly but significantly enhanced (1.9 times) the sensitivity to St-587 (control: 6.7±0.1; L-NAME: 7.0±0.05, $n=6$, $P<0.001$) without affecting the E_{max} (control: 21.1±1.5 mN; L-NAME: 22.5±1.8 mN, $n=6$). Thirty minutes exposure to the NO-synthase inhibitor in aortic rings did not modify the sensitivity (control: 5.0±0.2; L-NAME: 5.0±0.9, $n=7$), but drastically increased the E_{max} (control: 5.7±1.5 mN; L-NAME: 18.9±2.3 mN, $n=7$, $P<0.001$). No differences between E/[A] curves to phenylephrine or St-587 were observed when control experiments were run in parallel (results not presented).

Because contractile responses to α_1-adrenoceptor stimulation have been shown to be related to phosphoinositide turnover, the effect of St-587 on inositol phosphates formation in both blood vessels was studied. The influence of L-NAME on this response was also analyzed. In tail artery (Figure 3) but not in aorta (results not shown) St-587 induced inositol phosphate accumulation in a concentration-related manner. In rat aorta, St-587 failed to increase the inositol phosphates formation over the basal value (results not presented). The presence of L-NAME was without effect on basal (see legend of Figure 3) as well as on St-587-induced inositol phosphates formation in tail artery (Figure 3) and aorta (results not shown).

To test the idea that the differences observed in the contractile effect of St-587 between tail artery and aorta could be due to differences in the population of spare α_1-adrenoceptors, responses to the selective α_1-adrenoceptor agonist, phenylephrine, were studied with and without phenoxybenzamine.

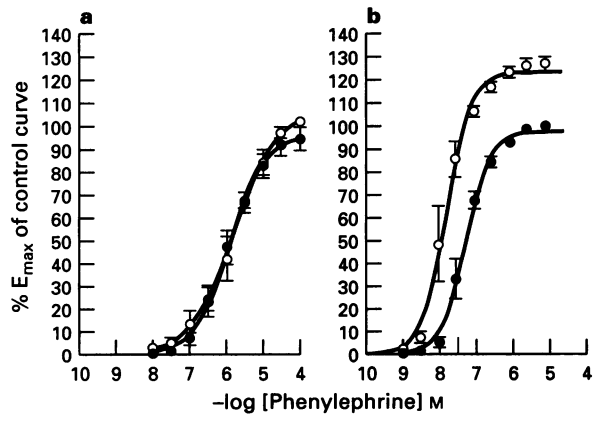

Figure 1 Concentration-response curves for phenylephrine-induced contraction in tail artery (a) and aorta (b) before (●) and after (○) incubation with L-NAME 30 μM. The lines drawn through the data are the results of pragmatic logistic curve fitting (see Methods). Each point represents the mean±s.e.mean of 6 experiments.

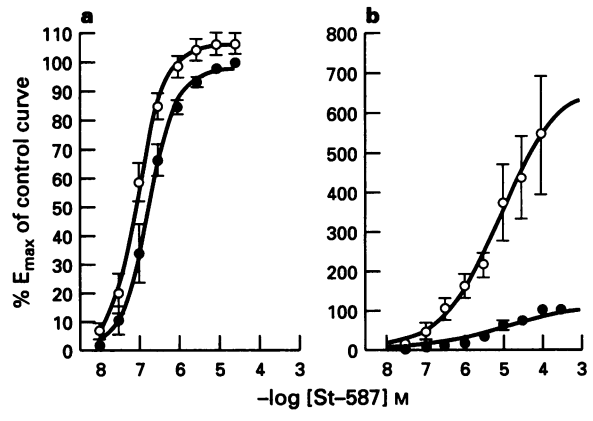

Figure 2 Concentration-response curves for St-587-induced contraction in tail artery (a) and aorta (b) before (●) and after (○) incubation with L-NAME 30 μM. The lines drawn through the data are the results of pragmatic logistic curve fitting (see Methods). Each point represents the mean±s.e.mean of 6-7 experiments.

The effects of phenoxybenzamine on phenylephrine E/[A] curves were examined in the tail artery (Figure 4a) and aorta (Figure 4b). Phenoxybenzamine treatment of the concentrations studied produced a non-parallel shift to the right of the E/[A] curves to phenylephrine with a depression of the maximum response that was more prominent in the aorta than in the tail artery (phenoxybenzamine 0.01 μM decreased by about 40% the maximum response in aorta, but in the tail artery, to produce a similar decrease a concentration of phenoxybenzamine three times higher (0.03 μM) was needed). Quantitative evaluation of the effects of phenoxybenzamine was carried out by the nested hyperbolic null method. Table 1 summarizes the parameters obtained for phenylephrine. All the E/[A] curves depicted in Figure 4 are included. Phenylephrine was 4.8 times more potent in aorta than in tail artery. The receptor occupancy was calculated for each concentration of phenylephrine and plotted as fractional occupancy against the fractional response (see legend of Figure 5). However, as shown, phenylephrine induced 50% of E_{max} in tail artery when only 1.1% of receptors were occupied but in the aorta around 21% of receptors were required to elicit half the maximum response.

In another set of experiments, the E/[A] curve to phenylephrine-induced contractions (Figure 6a) was again shifted to the right, in a non-parallel way by phenoxybenzamine (0.1 μM) and the E_{max} was reduced (control: 25.7±0.9 mN; POB: 7.8±0.8 mN, $n=8$). After the partial alkylation of α_1-adrenoceptors, L-NAME (30 μM) potentiated the concentration-response curve to phenylephrine. The E_{max} (16.8±1.1 mN, $n=8$) obtained was twice the maximum contraction obtained in presence of phenoxybenzamine alone. When the same experiments were run in parallel using PSS instead of L-NAME no difference was observed between the second and the third phenylephrine E/[A] curves, in the presence of phenoxybenzamine.

To evaluate whether the potentiating effect of L-NAME on phenylephrine-induced contractions observed after partial α_1-adrenoceptor inactivation could be due to an increase in the coupling process *via* inositol phosphate turnover, we studied the effect of L-NAME on inositol phosphate accumulation by phenylephrine, in the presence of phenoxybenzamine. The results obtained are shown in Figure 6b. Phenylephrine behaved as a full agonist producing a maximum accumulation of inositol phosphates formation (69.3±7.5% of ^{3}H-lipids, $n=4$) similar to the E_{max} obtained with noradrenaline (results not shown). The presence of phenoxybenzamine produced a non parallel shift to the right of the E/[A] curve for phenylephrine with a 50% reduction on the maximum accumulation (38.5±7.1% of ^{3}H-lipids, $n=4$). Nevertheless, L-NAME was unable to modify the responses to phenylephrine in presence of phenoxybenzamine either in sensitivity or maximum inositol phosphate formation.

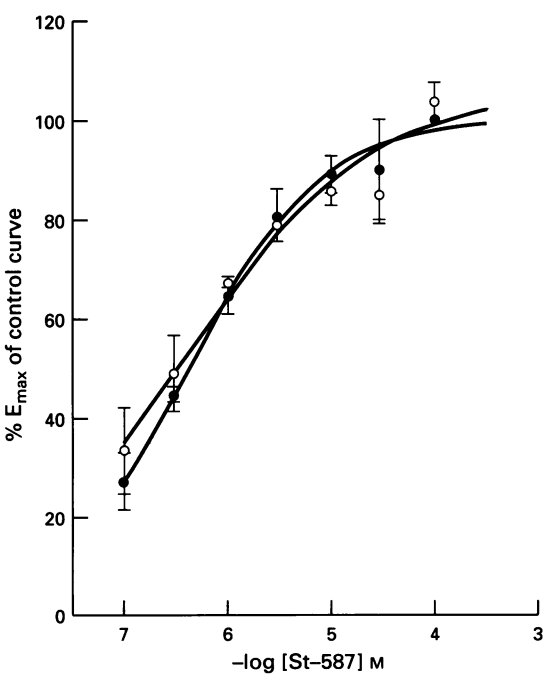

Figure 3 Concentration-response curves for St-587 induced accumulation of [^{3}H]-inositol phosphate formation in tail artery in absence (●) and in presence (○) of L-NAME 30 μM. The lines drawn through the data are the results of pragmatic logistic curve fitting (see Methods). Results are the mean±s.e.mean of 4 experiments performed in triplicate. Basal [^{3}H]-inositol phosphates average 1.501±0.158% of ^{3}H-lipids (control); 1.925±0.303% ^{3}H-lipids (L-NAME)

Discussion

The results of our study suggest that the extent of the influence of endothelium-released NO on α_1-adrenoceptor-mediated vasoconstriction greatly depends upon the postjunctional spare α_1-adrenoceptor population. In addition, our biochemical studies indicate that the potentiating effect of L-NAME is independent of intracellular calcium release *via* phosphatidylinositol turnover.

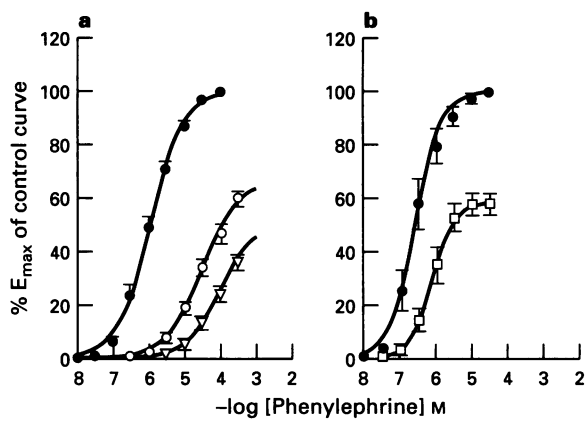

Figure 4 Effect of phenoxybenzamine pretreatment on phenylephrine-induced contraction in tail artery (a) and aorta (b). Average phenylephrine concentration-response curves (E/[A]) obtained before (●) and following a 10 min exposure to 0.01 μM (□), 0.03 μM (○) and 0.1 μM (▽) phenoxybenzamine are depicted. The lines drawn through the data are the results of pragmatic logistic fitting (see Methods). Each point represents the mean±s.e.mean of 10-19 experiments.

Table 1 Parameters estimates for phenylephrine obtained from the nested hyperbolic method

	Tail artery	*Aorta*
m	0.902±0.049	1.160±0.137
pEC_{50}	5.952±0.029	6.632±0.048
q_1	0.022±0.003	0.241±0.082
q_2	0.007±0.001	–
pK_a	4.014±0.122	6.003±0.252

For the analysis using the nested hyperbolic method only E/[A] curves obtained before and after treatment with phenoxybenzamine (aorta: 0.01 μM; tail artery: 0.03 μM and 0.1 μM) were considered. Values are mean±s.e.mean obtained as described in methods.

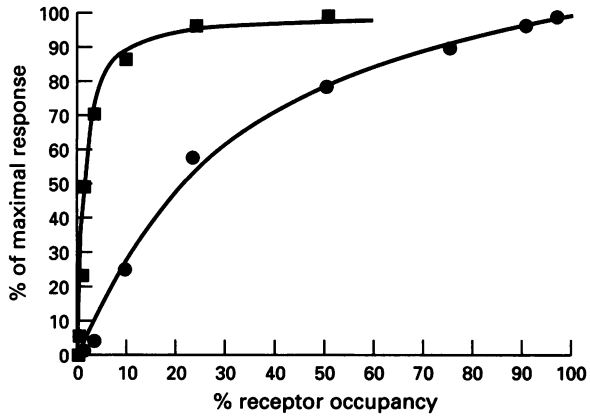

Figure 5 Plot of percentage of receptor occupancy versus percentage of response for the contractile effect of phenylephrine in tail artery (■) and aorta (●). The percentage occupancy of the receptors was calculated from the equation $[AR]/[R_T]=[A]/(K_A+[A])$ where $[AR]/[R_T]$ is the fractional receptor occupancy, K_A is the affinity constant calculated by the Nested hyperbolic method (tail artery: $K_A=90\,\mu M$; aorta: $K_A=1\,\mu M$) and [A] is the concentration of agonist phenylephrine.

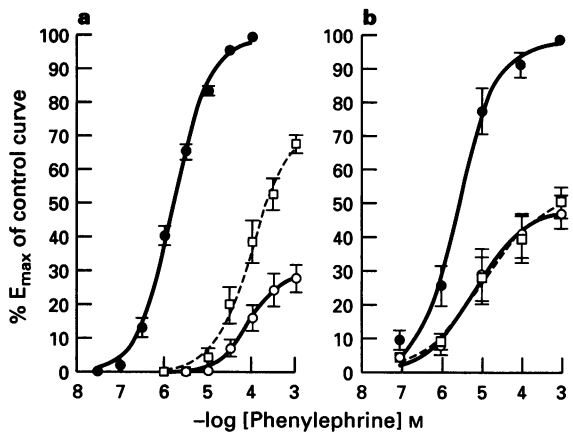

Figure 6 Concentration-response curves for phenylephrine-induced contraction (a) and inositol phosphates formation (b) in presence of saline (●), phenoxybenzamine 0.1 μM (○) and L-NAME 30 μM after phenoxybenzamine 0.1 μM (□). The lines drawn through the data are the results of pragmatic logistic curve fitting (see Methods). Results are the mean ± s.e.mean of 8 experiments for contraction and 4 experiments performed in triplicate for inositol phosphate formation studies.

Smooth muscle contraction induced by α-adrenoceptor agonists in rat aorta is mostly if not exclusively mediated by α_1-adrenoceptors (Ruffolo, 1985). Nevertheless, some (Weiss *et al.*, 1983; Vila *et al.*, 1993) but not all (Atkinson *et al.*, 1988) studies have suggested that α_1 and α_2-adrenoceptors are present in rat tail artery. Thus, in our experimental protocol, yohimbine (0.1 μM) was present to prevent the stimulation of α_2-adrenoceptors by agonists.

Studies in rat aorta (Egleme *et al.*, 1984; Alosachie & Godfraind, 1988; Topouzis *et al.*, 1991), suggest that the influence of endothelium on contractile responses to partial α_1-adrenoceptor agonists is greater than on responses to a full agonist. In our experiments, removal of endothelium increased the sensitivity and the maximum contraction to noradrenaline in the aorta without modifying the noradrenaline vasoconstriction in the tail artery from Sprague-Dawley rats (Tabernero & Vila, 1995).

Vascular endothelium releases several vasoconstrictor and vasodilator factors (Sanchez-Ferrer & Marín, 1990) that can modulate constrictor responses to agonists. In this sense, three factors released from tail artery endothelial cells (Thorin & Atkinson, 1994) that could modify vasoconstriction induced by agonists have been described. Thus, to avoid the influence of endothelial factors other than NO on α_1-adrenoceptor-mediated responses as well as to prevent the damage to smooth muscle cells that could particularly occur in a small blood vessel such as tail artery by mechanical removing of the endothelium, we investigated the effects of L-NAME, an inhibitor of NO synthase, on α_1-adrenoceptors responses in intact tail artery and aorta segments. The modulation of phenylephrine responses by L-NAME correlates with the results previously obtained using noradrenaline in the same blood vessels when endothelium was removed (Tabernero & Vila, 1995). The lack of NO shifted to the left the phenylephrine-induced contraction in the aorta but not in the tail artery, confirming a different modulation of α_1-adrenoceptor responses by NO, depending on the vessel studied. Our results in rat aorta agree with those of other authors (Topouzis *et al.*, 1991). However, the lack of NO modulation on phenylephrine tail artery responses contrast with reports of the depression by endothelium (Thorin & Atkinson, 1994) or the potentiation by L-NAME of noradrenaline responses (Reid *et al.*, 1991) observed in the same vessel. The differences in our results could be ascribed to the strain of rats (Sprague-Dawley *vs* Wistar) and/or to the experimental design used.The results obtained with phenylephrine (a full agonist) in presence of L-NAME prompted us to study the influence of the NO-synthase inhibitor on contractile responses induced by a partial agonist. St-587 contracted both vessels in a concentration-related manner. In the aorta, this compound behaved as a weak agonist resulting in a maximum contraction that was around 20% of phenylephrine or noradrenaline E_{max}. On strips of aorta (Beckering *et al.*, 1984) St-587 acted as a partial agonist showing a higher value of E_{max} and pEC_{50} than those obtained in our study. The greater contraction observed with aortic strips is likely to be due to a disruption of the endothelium produced in the preparation of strips. In any case, in tail artery rings St-587 produced an E/[A] curve with a value of E_{max} close to but significantly smaller than phenylephrine or noradrenaline maximum contraction indicating that this agonist behaved almost like a full agonist in this preparation. In agreement with our results, St-587 has been described as an α_1-adrenoceptor agonist with different activity depending on the tissue studied. In the epididymal portion of the rat vas deferens (Badia & Sallés, 1989) and in the rat and guinea-pig aorta (Beckering *et al.*, 1984) this compound acted as a partial agonist but in the rat anococcygeus muscle (Vila *et al.*, 1984) it behaved as a full agonist.

The blockade of NO synthesis, in the presence of L-NAME in aortic rings, potentiated about 3 times the maximum contractile effect of St-587. Nevertheless, the inhibition of NO synthesis resulted in a slight (0.28 log units) but significant increase of sensitivity with no modification of maximum contraction in tail artery. From our results it is clear that the influence of L-NAME on St-587-induced contraction is much more important in the aorta where the partial agonism is revealed, than in the tail artery where this compound acts as a full rather than a partial agonist. The huge difference in the α_1-adrenoceptor-mediated contraction by St-587 as well as in the influence of L-NAME on the response between tail artery and aorta seem to indicate a difference in the stimulus-response relationship. Results obtained with phenoxybenzamine on phenylephrine-induced contractions show that only 1.1% of the receptors in tail artery but 21% in aorta need to be occupied to attain half of the maximum contractile response. These results can be attributed to a different efficiency in the coupling mechanisms through stimulation of α_1-adrenoceptors indicating a higher functional receptor reserve for the tail artery than for the aorta.

Thus, the lack of effect of L-NAME on α_1-adrenoceptor-mediated responses observed in tail artery rings is likely to be due to its high population of spare α_1-adrenoceptors. To test

this hypothesis we studied whether the nitric oxide synthase inhibitor could potentiate the phenylephrine-induced responses once the population of adrenoceptors has been substantially decreased by phenoxybenzamine. In our experiments, the phenylephrine-induced contractile responses were clearly potentiated by L-NAME when phenoxybenzamine was present to decrease the population of α_1-adrenoceptors. This result clearly indicates that the extent of the influence of NO on α_1-adrenoceptor-mediated contractile responses in rat tail artery depends on the population of postjunctional spare α_1-adrenoceptors.

We have previously demonstrated in both blood vessels that noradrenaline increases in a concentration-dependent manner, the accumulation of inositol phosphates and that changes of contraction parallel changes of phosphatidylinositol turnover (Vila *et al.*, 1993; Tabernero & Vila, 1995). St-587 induced an accumulation of inositol phosphates in a concentration-dependent manner in tail artery but not in aorta, results that partly agree with those obtained in contractile studies where the ability of St-587 to produce contraction was greater in the tail artery than in the aorta. Stimulation of α_1-adrenoceptors in rat aorta activates extracellular Ca^{2+} influx and/or intracellular Ca^{2+} release for contraction (Cauvin & Malik, 1984). In rat tail artery α_1-adrenoceptors mediate a fast and transient increase in intracellular Ca^{2+} which is probably the result of the mobilization of intracellular calcium and Ca^{2+} influx through receptor-operated channels (Li *et al.*, 1993). In addition, direct studies in tail artery rings using Fura 2 (Thorin & Atkinson, 1994) or indirect studies using inositol phosphate accumulation (Labelle & Murray, 1990; Vila *et al.*, 1993) have demonstrated that increases of intracellular calcium and contractions occur with α_1-adrenoceptor agonists. The results of these studies suggest that, in rat tail artery, contraction by an α_1-adrenoceptor agonist depends heavily but not exclusively on intracellular calcium increase. Studies in the aorta have led us to speculate that the component of the α_1-adrenoceptor-mediated response that is produced by the influx of extracellular Ca^{2+} has an extremely high coupling giving rise to an α_1-adrenoceptor reserve for this component of Ca^{2+} utilization, whereas the intracellular component has a low efficiency of coupling leading to a low or nonexistent α_1-adrenoceptor reserve (Chiu *et al.*, 1987). Therefore, it will explain why St-587, a partial agonist for contraction, did not stimulate the phosphoinositide hydrolysis in rat aorta. The fact that this compound was able to stimulate the inositol phosphates formation in rat tail artery could be due either to the behaviour of St-587, a strong agonist for contraction, or to the existence of α_1-adrenoceptor reserve for inositol phosphate formation (Fox & Friedman, 1986).

The increase in the efficacy of α_1-adrenoceptor-mediated responses due to L-NAME observed in aortic contractile experiments with St-587 or in the tail artery with phenylephrine in the presence of phenoxybenzamine, could result in an enhancement of coupling between α_1-adrenoceptors and phospholipase C or to an alteration in entry of extracellular calcium, or both. It is widely accepted that NO increases the formation of intracellular cyclic GMP responsible for its vasodilator effect (Rapoport *et al.*, 1983). Furthermore, it has also been reported that cyclic GMP diminishes the accumulation of inositol phosphates induced by stimulation of α_1-adrenoceptors (Rapoport, 1986; Hirata *et al.*, 1990) in rat aorta. Hence, we thought that a blockade of NO synthase by L-NAME could result in a potentiation of agonist-induced inositol phosphates accumulation due to a decrease in cyclic-GMP formation. An increase of coupling in α_1-adrenoceptor-induced contraction by removal of endothelium has been suggested in the rat aorta (Alosachie & Godfraind, 1988). However, L-NAME was unable to modify the inositol phosphates accumulation by St-587 in aorta, suggesting that the mechanisms responsible for the observed potentiation of α_1-adrenoceptor-mediated contractions are independent of phosphatidylinositol turnover. Therefore we are tempted to speculate that the potentiation of St-587 vasoconstriction by L-NAME observed with aortic rings could be explained by an increase in the efficiency of coupling for α_1-adrenoceptor stimulation linked to extracellular Ca^{2+}. In the rat tail artery, in spite of the great dependence on phosphatidylinositol hydrolysis for contraction by St-587 and phenylephrine as well as the existence of receptor reserve for phosphatidylinositol turnover, no modification in inositol phosphate formation by L-NAME in the absence or presence of phenoxybenzamine was observed. An alternative explanation could be an increase in the efficiency of coupling to extracellular Ca^{2+} as suggested for the aorta or to an increase in intracellular Ca^{2+} release from sources other than the sarcoplasmic reticulum. Further studies should be pursued to elucidate the intracellular mechanisms responsible for the enhancement by L-NAME of α-adrenoceptor-mediated contraction in the tail artery.

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