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Exposure and characterization of the action of noradrenaline at dopamine receptors mediating endothelium-independent relaxation of rat isolated small mesenteric arteries

[1]Pieter H. Van der Graaf, Pramod R. Saxena, *Nigel P. Shankley & *James W. Black

Department of Pharmacology, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands and *Department of Analytical Pharmacology, King's College School of Medicine and Dentistry, 123 Coldharbour Lane, London SE5 9NU

1 Previously, we reported that noradrenaline (NA), in addition to its α_1-adrenoceptor-mediated contractile effect, may relax the rat small mesenteric artery (SMA) in order to account for steep Schild plots obtained with compounds classified as α_1-adrenoceptor antagonists. In this study, a relaxant action of NA has been exposed in the rat isolated, endothelium-denuded SMA precontracted by the thromboxane A_2-mimetic, U46619.

2 NA, but not the selective α_2-adrenoceptor agonist, UK14304, produced concentration-dependent contraction of the SMA ($pEC_{50} = 5.7 \pm 0.1$). After precontraction with 0.1 μM U46619, 10 nM - 30 μM NA produced a further contraction ($pEC_{50} = 6.1 \pm 0.2$), while higher concentrations of NA produced small, but significant, relaxant responses.

3 In the presence of 1 μM prazosin, 0.1 - 30 μM NA produced concentration-dependent relaxation ($pIC_{50} = 5.9 \pm 0.1$) after precontraction with 0.1 μM U46619. The NA relaxation concentration-effect curve was completely inhibited by 1 μM of the β_1/β_2-adrenoceptor antagonist, timolol. However, when the concentration of prazosin was increased by 10 fold (10 μM), NA once again produced concentration-dependent relaxation ($pIC_{50} = 4.5 \pm 0.2$). This relaxation concentration-effect curve was not blocked by a 10 fold higher concentration of timolol (10 μM), nor by the presence of idazoxan (10 μM), cyanopindolol (10 μM), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), indomethacin (10 μM) or sulpiride (1 μM). However, haloperidol (10 μM) and (±)-SCH-23390 (10 nM) produced significant inhibition of the relaxation, suggesting the involvement of dopamine D_1 receptors.

4 Dopamine also produced concentration-dependent relaxation following U46619 precontraction ($pIC_{50} = 5.4 \pm 0.1$) which was significantly inhibited by haloperidol and (+)-SCH-23390. Pretreatment with 10 μM phenoxybenzamine for 60 min produced a significant inhibition of the dopamine and NA relaxation curves and application of the operational model of agonism yielded estimates of the affinity ($pK_A = 5.3 \pm 0.2$ and 4.4 ± 0.2) and efficacy ($\log \tau = 0.06 \pm 0.11$ and 0.01 ± 0.10) for dopamine and NA, respectively, at D_1receptors.

5 HV723 (0.1 and 1 μM), a ligand that yielded a Schild plot slope parameter of unity as an antagonist of NA in the contractile assay, produced concentration-dependent inhibition of the NA-mediated relaxation ($pA_2 \sim 8$).

6 The results of this study indicate that NA can activate D_1receptors mediating relaxation in the rat SMA at concentrations which were encountered in our previous receptor classification experiments using competitive α_1-adrenoceptor antagonists.

Keywords: Noradrenaline; dopamine; rat small mesenteric artery; α_1-adrenoceptors; β-adrenoceptors; dopamine D_1 receptors; Schild analysis

Introduction

Noradrenaline (NA) is generally accepted to contract resistance arteries by acting at α-adrenoceptors (see Mulvany & Aalkjaer, 1990). However, recently we found that a series of α_1-adrenoceptor antagonists did not produce the expected behaviour for competitive antagonism of NA-induced contraction of the rat isolated small mesenteric artery (SMA; Van der Graaf *et al.*, 1995b). Having rejected the usual explanatory models for steep Schild plots (i.e. insufficient antagonist incubation time, antagonist uptake and functional antagonism) we speculated that they might be due to an additional relaxant action of NA (Van der Graaf *et al.*, 1995b). β_1/β_2-Adrenoceptors, which have previously been shown to mediate relaxation in the SMA (Graves & Poston, 1993), appeared not to be involved because the experiments were performed in the presence of timolol (6 μM, ~100 fold its affinity for β_1/β_2-adrenoceptors; see Hieble, 1991). Therefore, in an attempt to expose any relaxant action of NA, we have examined the effects of NA on precontracted SMA. We now show that, in the presence of α_1- and β-adrenoceptor blockade, NA does indeed inhibit the contraction of the SMA to the thromboxane A_2-mimetic, U46619, and to 5-hydroxytryptamine (5-HT) via an endothelium-independent pathway.

A preliminary account of these data was presented to the British Pharmacological Society (Van der Graaf *et al.*, 1995a).

Methods

Rat isolated small mesenteric artery preparation

Male Wistar rats (225 - 300 g) were killed by cervical dislocation and the mesentery was removed and placed in ice-cold modified Krebs-Henseleit solution (KHS) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, KCl, 4.7,

[1] Author for correspondence.

KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5.5, $CaCl_2$ 2.5 and ethylenediaminetetra-acetic acid (EDTA) 0.026. Six arterial trees were dissected from each mesenteric vascular bed and cleared from surrounding adipose tissue. From each arterial tree, a ~2 mm ring segment was mounted in a small vessel myograph (J.P. Trading, Aarhus, Denmark) with separated 6 ml organ baths (thermostatically controlled at 37±0.5°C, containing modified KHS and continuously gassed with 95% O_2 and 5% CO_2) as described by Mulvany & Halpern (1977). The endothelium was removed by gentle rubbing of the intimal surface with a thin, scoured, metal wire. Tissue responses were continuously measured as changes in isometric tension and displayed on potentiometric chart recorders.

Experimental protocol

Following a 30 min stabilization period, the internal diameter of each vessel was set to a tension equivalent to 0.9 times the estimated diameter at 100 mmHg effective transmural pressure ($l_{100}=200\pm4$ μm, $n=125$) according to the standard procedure of Mulvany & Halpern (1977). After a further 15 min stabilization period, a calibration contraction (6.9±0.3 mN) was obtained to 10 μM NA in each tissue and the absence of the endothelium was then confirmed by the lack of a response to 10 μM 5-methylfurmethide, the acetylcholine M-receptor agonist. After a 15 min washout period, tissues were incubated for 60 min with either antagonist or vehicle. Each vessel was then precontracted with a single concentration (0.1 μM) of U46619, the thromboxane A_2-mimetic. After a response plateau was achieved, single NA, phenylephrine (PE) and dopamine concentration-effect (E/[A]) curves were obtained by cumulative dosing at half-log unit concentration increments. Effects were expressed as a percentage of the precontraction response to U46619 and are given as the mean ±s.e.mean. In the experiments in which agonist E/[A] curves were obtained in the absence of precontraction, the response to 0.1 μM U46619 was measured after completion of the E/[A] curve and washout of the agonist. Cocaine (30 μM) was present in all experiments to block neuronal uptake. Extraneuronal uptake did not appear to play a role in the SMA assay because it was shown in preliminary experiments that 10 μM corticosterone had no significant effect on the E/[A] curve of NA as a contractile agent in the absence of precontraction (data not shown).

Analysis

Estimation of Hill equation parameters Individual NA contraction E/[A] data, in the absence and presence of precontraction (E_{prec}), were fitted to the following form of the Hill equation using an iterative, least-squares method:

$$E = E_{prec} + \frac{(\alpha - E_{prec})[A]^{n_H}}{EC_{50}^{n_H} + [A]^{n_H}} \quad (1)$$

to provide estimates of midpoint slope (n_H), midpoint location (EC_{50}, estimated as a logarithm) and upper asymptote (α). Individual agonist relaxation curves obtained after precontraction were fitted to the following form of the Hill equation:

$$E = 100\% - \frac{\alpha \times [A]^{n_H}}{IC_{50}^{n_H} + [A]^{n_H}} \quad (2)$$

where IC_{50} is the midpoint location of the relaxation E/[A] curve. The effect of drug treatment on these parameters was assessed by one-way analysis of variance (ANOVA) or Student's t test, as appropriate. Values of $P<0.05$ were considered to be significant.

Estimation of affinity and efficacy Individual NA and dopamine relaxation curve data, in the absence and presence of phenoxybenzamine (PBZ) pretreatment, were fitted simultaneously to the following form of operational model of agonism (Black & Leff, 1983; Leff *et al.*, 1990) using the AR module (derivative-free non-linear regression) of the BMDP statistical software package (Dixon *et al.*, 1990):

$$E = 100\% - \frac{E_m \times \tau^n \times [A]^n}{(K_A + [A])^n + \tau^n \times [A]^n} \quad (3)$$

to provide single estimates of the maximum effect achievable in the system (E_m), the slope index for the receptor occupation-effect relation (n), the dissociation equilibrium constants (estimated as negative logarithm, that is pK_A) for NA and dopamine and individual estimates of the transducer ratio (τ, estimated as logarithm). The individual estimates of log τ for the control curves were then used to calculate a mean log τ ± s.e.mean for NA and dopamine. Standard errors on E_m, n and pK_A were calculated using the "scaling-up" method described by Leff *et al.* (1990).

Compounds

Compounds were obtained from the following sources: cocaine hydrochloride, dopamine hydrochloride, 5-hydroxytryptamine hydrochloride (5-HT), indomethacin, N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME), (-)-noradrenaline bitartrate (NA), (-)-phenylephrine hydrochloride (PE), prazosin hydrochloride, U46619 (9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin $F_{2\alpha}$): Sigma Chemie, The Netherlands; idazoxan hydrochloride, phenoxybenzamine hydrochloride (PBZ), SCH-23390 (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) and UK14304 (5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2yl)-6-quinoxalinamine): Research Chemicals Incorporated, U.S.A.; haloperidol: Janssen Biotech. N.V., Belgium; (±)-sulpiride: Pharmexport B.V., The Netherlands; 5-methylfurmethide iodide: Wellcome Research Laboratories Ltd., U.K.; timolol maleate: Merck, Sharp & Dohme, U.K.; HV723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy) ethyl) amino) propyl) benzene-aceto-nitrile fumarate: a gift from Professor dr I. Muramatsu, Fukui Medical School, Japan; (-)-tamsulosin (previously known as YM617): a gift from Yamanouchi Pharmaceutical Co. Ltd., Japan.

NA, PE, dopamine and 5-HT were dissolved and diluted in stoichiometric, aqueous ascorbic acid solution. UK14304 was initially dissolved in 0.1 M hydrochloric acid to give 1 mM stock solutions and subsequently diluted in distilled water. Indomethacin, haloperidol, sulpiride and PBZ were dissolved in absolute ethanol. Prazosin was dissolved in 50% ethanol. All other drugs were dissolved in distilled water. NA, dopamine, PE and 5-HT stock solutions were made up each day. All other drug stock solutions were stored below −20°C and diluted on the day of the experiment. The maximum volume of drug solution administered to the 6 ml organ baths did not exceed 500 μl, corresponding to ~8% of the bath volume. Neither the vehicles nor any of the treatments with antagonists were found to produce significant effects on basal tone or on the precontraction response to 0.1 μM U46619.

Results

Noradrenaline contractile responses

NA (10 nM−300 μM), but not the selective α_2-adrenoceptor agonist, UK14304 (10 nM−1 mM), produced concentration-dependent contraction of the SMA (Figure 1). After precontraction with 0.1 μM U46619, which produced a sustained contraction of 87±13% ($n=4$) compared to the 10 μM NA sighting response, 10 nM−30 μM NA produced a further contraction while higher (0.1−1 mM) concentrations of NA produced small, but significant, relaxatory responses (Figure 1). The contractile E/[A] data obtained in the absence and presence of U46619 were fitted to Equation (1) and comparison of the parameters estimated revealed no significant dif-

ferences between the midpoint locations ($pEC_{50}=5.7\pm0.1$ and 6.1 ± 0.2, respectively, $P>0.1$, d.f.$=6$) and upper asymptotes ($\alpha=118\pm11$ and $134\pm5\%$ of the response to 0.1 μM U46619, respectively, $P>0.2$). However, the NA E/[A] curve obtained in the presence of U46619 was significantly steeper than the control curve ($n_H=1.48\pm0.07$ and 0.98 ± 0.07, respectively, $P<0.005$).

Noradrenaline relaxant responses

An attempt was then made to expose a relaxant action of NA by blocking the α_1-adrenoceptor-mediated contractile responses. Thus, in the presence of prazosin (1 μM, ~300 fold its affinity for α_1-adrenoceptors in the SMA; Van der Graaf *et al.*, 1995b), 0.1–30 μM NA produced significant inhibition of the U46619 response ($\alpha=89\pm5\%$, $pIC_{50}=5.9\pm0.1$, $n_H=1.2\pm0.1$, $n=5$). Higher (0.1–1 mM) concentrations of NA produced contractile responses which ultimately reversed the relaxation (Figure 2). This NA relaxant concentration-effect curve was completely inhibited by 1 μM timolol, the non-selective β_1/β_2-adrenoceptor antagonist (Figure 2). When the concentration of prazosin was increased by 10 fold (10 μM), in order to enlarge the experimental window for studying relaxation, NA once again produced concentration-dependent relaxation (Figure 2). This relaxation curve ($\alpha=73\pm10\%$, $pIC_{50}=4.5\pm0.2$, $n_H=1.2\pm0.2$, $n=4$) was not affected even by a 10 fold higher concentration (10 μM) of timolol, implying that β_1/β_2-adrenoceptors were not involved in this particular relaxatory response to NA (Figure 2). Furthermore, PE (1 μM–1 mM) did not produce a significant response following precontraction with 0.1 μM U46619 in the presence of 10 μM prazosin and 10 μM timolol (maximum relaxation $=3\pm4\%$, maximum contraction $=3\pm2\%$, $n=4$), suggesting that the relaxation was not mediated by α_1-adrenoceptors.

Other possible mechanisms for the relaxation were investigated. The relaxation did not appear to be mediated by α_2 or 'atypical' β-adrenoceptors or by the synthesis of nitric oxide (NO) or prostaglandins, because idazoxan (10 μM, ~4000 fold its affinity for α_2-adrenoceptors in rat vas deferens; Chapleo *et al.*, 1981), cyanopindolol (10 μM, ~400 fold its affinity for 'atypical' β-adrenoceptors in guinea-pig ileum; Blue *et al.*, 1989), the NO synthase inhibitor, L-NAME (100 μM, ~200 fold IC_{50} value for inhibition of endothelium-dependent relaxation of rat aorta; Rees *et al.*, 1990) and indomethacin (10 μM; IC_{50} value for inhibition of prostaglandin synthesis in various *in vitro* assays ~1 μM; see Shen, 1979) had no significant effect (Table 1).

The relaxation did not appear to be due to specific inhibition of the response to U46619, because NA also relaxed SMAs which were precontracted ($71\pm6\%$ compared to the 10 μM NA sighting response, $n=4$) with 1 μM 5-HT in the

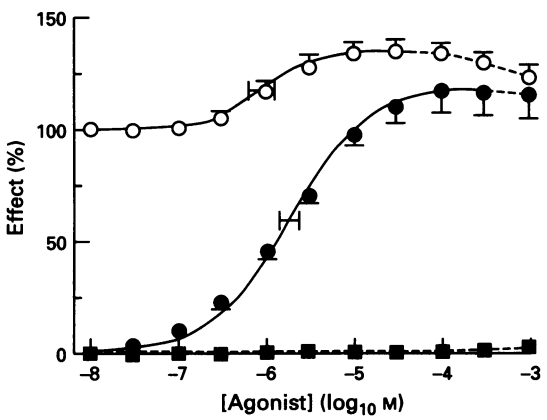

Figure 1 Contractile concentration-effect curves obtained on the rat small mesenteric artery to noradrenaline (●) and UK14304 (■) and to noradrenaline following precontraction with 0.1 μM U46619 (○). The solid lines shown superimposed on the mean experimental data points ($n=4$, ±s.e.mean) were simulated using Equation 1 (see text for parameter estimates). Horizontal error bars (s.e.mean) are shown on the location of the mean EC_{50} estimates. The supramaximal data points that were not included in the fitting procedure are shown connected by dashed lines. Contractile effects are expressed as percentage of the response to 0.1 μM U46619.

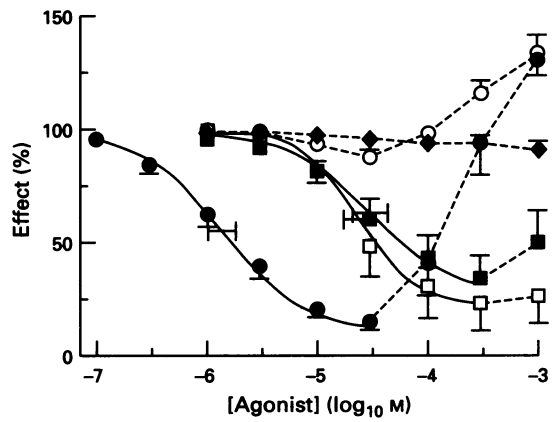

Figure 2 Relaxation concentration-effect curves obtained on the rat small mesenteric artery to noradrenaline following precontraction with 0.1 μM U46619 in the presence of 1 μM prazosin (●), 1 μM prazosin and 1 μM timolol (○), 10 μM prazosin and 1 μM timolol (■) and 10 μM prazosin and 10 μM timolol (□). The solid lines shown superimposed on the mean experimental data points ($n=4$–5, ±s.e.mean) were simulated using Equation 2 (see text for parameter estimates). Horizontal error bars (s.e.mean) are shown on the location of the mean IC_{50} estimates. The data points that were not included in the fitting procedure are shown connected by dashed lines. The response to the vehicle (ascorbic acid) in the presence of 10 μM prazosin and 10 μM timolol is also shown (◆, $n=4$). Relaxant effects are expressed as percentage of the precontraction response to 0.1 μM U46619.

Table 1 The effect of idazoxan, cyanopindolol, L-NAME and indomethacin on the midpoint location (pIC_{50}), maximum relaxation (α) and Hill slope (n_H) of noradrenaline inhibition curves obtained on rat small mesenteric arteries precontracted with U46619 (0.1 μM) in the presence of timolol (10 μM) and prazosin (10 μM)

Treatment	*pIC_{50}*	*α (% relaxation)*	*n_H*	n^1
Control	4.6±0.1	78±11	1.7±0.3	4
Idazoxan (10 μM)	4.9±0.2	68±13	1.4±0.2	4
Cyanopindolol (10 μM)	4.8±0.1	72±10	1.4±0.1	4
L-NAME (100 μM)	4.6±0.1	77±3	1.3±0.1	4
Indomethacin (10 μM)	4.8±0.1	92±6	1.3±0.2	4
ANOVA	$P>0.3$	$P>0.4$	$P>0.4$	

[1] Number of observations.

presence of 10 μM prazosin and 10 μM timolol ($\alpha = 73 \pm 10\%$, $pIC_{50} = 5.0 \pm 0.1$, $n_H = 0.9 \pm 0.2$). Furthermore, in the presence of 10 μM timolol, NA also inhibited the U46619 response when 10 μM tamsulosin (a phenethylamine analogue, pA_2 for α_1-adrenoceptor-mediated contraction in SMA ~9.8; Van der Graaf *et al.*, 1995b) instead of prazosin (a quinazoline) was used to block the α_1-adrenoceptor-mediated contraction ($\alpha = 72 \pm 10\%$, $pIC_{50} = 4.4 \pm 0.2$, $n_H = 1.2 \pm 0.1$, $n = 4$). This suggests that the relaxation was not due to a specific property of α_1-adrenoceptor antagonists from one chemical class.

Effects of dopamine antagonists

Wanstall & O'Donnell (1989) have suggested that NA produces relaxation in the rat perfused mesenteric preparation by acting at dopamine D_1 receptors. Therefore, we have investigated whether dopamine receptors were also involved in the relaxation of the SMA to NA in the presence of α_1 and β-adrenoceptor blockade. After precontraction with 0.1 μM U46619 in the presence of 10 μM prazosin and 10 μM timolol, a concentration-dependent relaxation was obtained to dopamine ($\alpha = 71 \pm 9\%$, $pIC_{50} = 5.4 \pm 0.1$, $n_H = 1.0 \pm 0.1$, $n = 4$; Figure 3a). The dopamine-induced relaxation was significantly inhibited by the non-selective dopamine receptor antagonist, haloperidol (10 μM), and by the selective D_1 receptor antagonist, SCH-23390 (10nM, ~20 fold its K_i value for dopamine-stimulated cyclic AMP formation in cultured smooth muscle cells obtained from rat mesenteric artery; Hall *et al.*, 1993b; Figure 3a). It was not possible to test the basic criteria for competitive antagonism because of the limited experimental window. In contrast, sulpiride, at a concentration (1 μM) ~100 times higher than its reported K_B value for D_2 receptors in mouse vas deferens (Martin *et al.*, 1993), had no significant effect (Figure 3a). The NA-induced relaxation was also inhibited by 10 μM haloperidol and 10 nM SCH-23390 but not by 1 μM sulpiride (Figure 3b). Therefore, these experiments suggest that, in the presence of α_1 and β-adrenoceptor blockade, NA and dopamine both act at D_1-receptors to inhibit the response to U46619.

Estimation of affinity and efficacy of noradrenaline and dopamine at putative D_1 receptors with phenoxybenzamine

Pretreatment of SMAs with 10 μM PBZ for 60 min produced a significant rightward shift and depression of the maximum of the NA and dopamine relaxation curves (Figure 4). Individual control and PBZ-treated NA and dopamine E/[A] curves were fitted simultaneously to the operational model of agonism (Equation 3). The parameter estimates are summarised in Table 2 and were used to simulate the curves shown superimposed on the experimental data points in Figure 4. As shown in Table 2, dopamine was found to display an approximately 10 fold higher affinity than NA, however, the efficacy ($\log \tau$) estimates for the dopamine and NA control curves were not significantly different ($P > 0.5$, d.f. = 6).

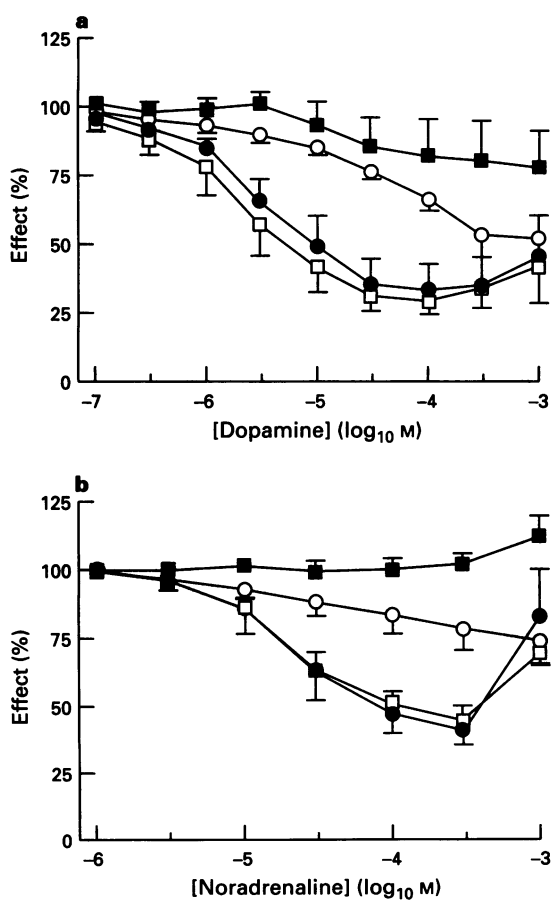

Figure 3 Relaxation concentration-effect curves obtained on the rat small mesenteric artery in the presence of 10 μM prazosin and 10 μM timolol to (a) dopamine and (b) noradrenaline following precontraction with 0.1 μM U46619 in the absence (●) and presence of 10 μM haloperidol (○), 10 nM SCH-23390 (■) and 1 μM sulpiride (□). Effects ($n = 4$, mean ± s.e.mean) are expressed as percentage of the precontraction response to 0.1 μM U46619.

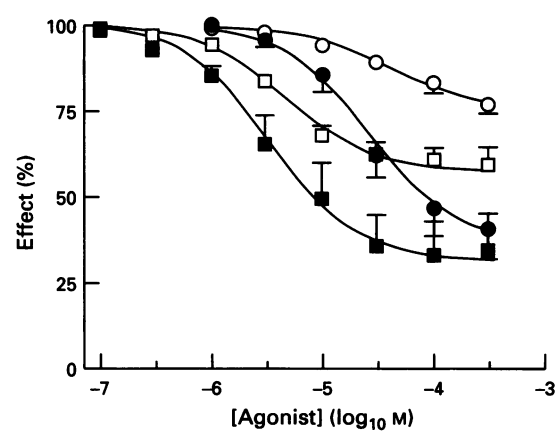

Figure 4 Noradrenaline (○,●) and dopamine (□,■) relaxation concentration-effect curves obtained on the rat small mesenteric artery in the presence of 10 μM prazosin and 10 μM timolol following 60 min pretreatment with vehicle (closed symbols) and 10 μM phenoxybenzamine (open symbols). The solid lines shown superimposed on the mean experimental data points ($n = 4$, ± s.e.mean) were simulated using the operational model of agonism (Equation 3) with the parameter estimates summarised in Table 2. Effects are expressed as percentage of the precontraction response to 0.1 μM U46619.

Table 2 Operational model of agonism parameter estimates (mean ± s.e.) for the interaction of noradrenaline and dopamine at D_1 receptors mediating relaxation of the U46619-precontracted rat small mesenteric artery

	Dopamine	*Noradrenaline*
$\log \tau_{control}$	0.06 ± 0.11	0.01 ± 0.10
$\log \tau_{PBZ\text{-}treated}$	−0.23 ± 0.04	−0.48 ± 0.05
pK_A	5.3 ± 0.2	4.4 ± 0.2

The results were obtained from 4 experiments. $E_M = 127 \pm 26\%$, $n = 1.3 \pm 0.3$.

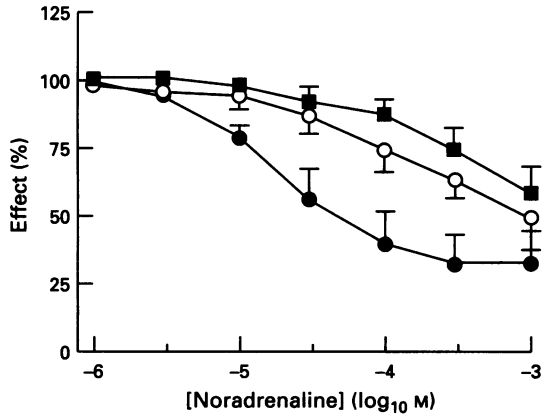

Figure 5 Relaxation concentration-effect curves to noradrenaline obtained on the rat small mesenteric artery in the presence of 10 μM prazosin and 10 μM timolol following precontraction with 0.1 μM U46619 in the absence (●) and presence of 0.1 (○) and 1 μM HV723 (■). Effects ($n=4$, mean ± s.e.mean) are expressed as percentage of the precontraction response to 0.1 μM U46619.

Effect of HV723 on the relaxation to noradrenaline

Our hypothesis is that the relaxant action of NA dopamine D_1 receptors is responsible for the steep Schild plots obtained in our previous study with some of the compounds classified as α_1-adrenoceptor antagonists (Van der Graaf *et al.*, 1995b). If this is valid, those antagonists which produced a Schild plot slope of unity would be expected to block the action of NA at this receptor. This was investigated by use of HV723, a ligand previously classified as a selective α_1-adrenoceptor antagonist ($pA_2=9.2$ in rat aorta; Muramatsu *et al.*, 1990), which behaved as a simple competitive antagonist of NA in the contractile SMA assay ($pK_B=8.96\pm0.08$; Van der Graaf *et al.*, 1995b). HV723 produced a concentration-dependent inhibition of the relaxation produced by NA at concentrations (0.1 and 1 μM) which were approximately 100 and 1000 fold higher than its affinity measured in the contractile assay (Figure 5). Although, due to the limited experimental window, it was not possible to test whether HV723 was behaving as a competitive antagonist, the shift obtained with 0.1 μM was consistent with a pA_2 value of ~8.

Discussion

Previously, we considered the possibility that NA can also express an inhibitory action in the rat isolated, endothelium-denuded, SMA at concentrations encountered when contractile E/[A] curves were rightward shifted in the presence of α_1-adrenoceptor antagonists (see Introduction; Van der Graaf *et al.*, 1995b). In the present study, in accord with this hypothesis, NA was shown to relax the precontracted SMA under conditions where α_1-adrenoceptors mediating contraction were blocked. The analysis revealed that there were two components to this relaxant action of NA. The most potent action, perhaps as expected, was mediated by β-adrenoceptors as judged by its abolition by selective concentrations of timolol (Figure 2). The second component was not blocked by selective concentrations of inhibitors of several other recognised mediators of vascular relaxation (Table 1) but was blocked by the selective dopamine receptor antagonist, haloperidol (Figure 3). The finding that SCH-23390, but not sulpiride, blocked the relaxation suggests that dopamine receptors of the D_1 class were involved (see Watson & Girdlestone, 1995).

The existence of dopamine receptors mediating vascular relaxation is well established (see Hughes & Sever, 1989). Indeed, several groups have reported the presence of D_1 receptors in the rat mesentery (e.g. Nichols & Hiley, 1985; Dupont *et al.*, 1987; Wanstall & O'Donnell, 1989; Perretti *et al.*, 1990; Amenta *et al.*, 1991; Hall *et al.*, 1993a,b). Initially, however, we did not consider dopamine receptors because Heesen & De Mey (1990) had shown that dopamine was able to relax KCl-precontracted rat SMA and in preliminary experiments we found that NA could not relax tissues contracted with KCl (data not shown). Our experiments differed from those of Heesen & De Mey (1990) in that our preparations were denuded of endothelium and a higher KCl concentration was used (75 compared with 30 mM). We do not know if these conditions could account for the failure to see inhibition of KCl-induced contraction.

Although Goldberg *et al.* (1978) concluded that NA was inactive at dopamine receptors in the canine renal vasculature, Crooks & Martin (1979) and Vanderheyden *et al.* (1986) described an action of NA at dopamine receptors in rabbit splenic artery and bovine retina homogenate, respectively. Indeed, Wanstall & O'Donnell (1989) have already reported that in the rat perfused mesentery preparation, precontracted with KCl and vasopressin, relaxation produced by NA is mediated by both β-adrenoceptors and D_1 receptors.

The contractile NA curve, presumed to be α_1-adrenoceptor mediated, had a midpoint location (pEC_{50}) value of 5.7 which was not significantly different from the location of the β-adrenoceptor-mediated curve obtained following precontraction by U46619 in the presence of α_1-adrenoceptor blockade ($pIC_{50}=5.9$). Therefore, notwithstanding the possibility of some underestimation of the potency of NA at the β-adrenoceptors due to functional antagonism by the precontractile agent, NA appeared to be approximately equipotent at these receptors. In contrast, NA was approximately 10 fold less potent at the D_1 receptor. The application of the operational model of agonism to the data obtained following pretreatment with PBZ, which has previously been shown to bind irreversibly to D_1 receptors in rat mesenteric vascular smooth muscle cells (Hall *et al.*, 1993a), provided estimates of the affinity (pK_A) and efficacy (log τ) parameters for NA and dopamine. The values obtained (Table 2) indicate that NA expresses the same efficacy at the D_1 receptor as dopamine itself and that the difference in potency of dopamine and NA at the D_1 receptor can be attributed simply to NA having an approximately 10 fold lower affinity for the receptor. Furthermore, comparison of the pK_A estimate for NA at the D_1 receptor with previously reported pK_A values at α_1-adrenoceptors mediating contraction of the SMA (5.4, Nyborg & Bevan, 1988; 5.6, Bevan *et al.*, 1989; 5.8, Nielsen & Mulvany, 1990) suggests that the affinity of NA for D_1 receptors may only be 10 fold lower than for α_1-adrenoceptors.

In conclusion, the results of this study show that NA can activate D_1 receptors mediating relaxation in the rat SMA at concentrations which were applied in our previous receptor classification experiments using competitive α_1-adrenoceptor antagonists (Van der Graaf *et al.*, 1995b). The original hypothesis was that an additional relaxant action of NA was responsible for steep Schild plots. The finding that HV723, but not prazosin or tamsulosin, blocked this action at concentrations which were used to estimate its affinity at α_1-adrenoceptors conforms with the hypothesis because of the three, only HV723 produced a Schild plot with unit slope in the contractile assay. Therefore, we conclude that the steep Schild plots obtained in our previous study may have been due to an action of NA at D_1 receptors mediating relaxations. Such a dual action of an agonist within one assay appears to be another way in which steep Schild plots can be obtained.

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