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Investigation of the subtypes of α_1-adrenoceptor mediating contractions of rat vas deferens

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1 The subtypes of α_1-adrenoceptor mediating contractions of rat vas deferens to endogenous and exogenous noradrenaline and to the exogenous agonists methoxamine, phenylephrine and A61603 have been examined.

2 The effects of antagonists on the shape of concentration-response curves, both tonic and phasic, to the four agonists were analysed. Prazosin produced parallel shifts in all cases. Particularly for RS 17053 against noradrenaline, there was some evidence for a resistant component of the agonist response. High concentrations of RS 17053 (1–10 μM) virtually abolished tonic contractions but phasic contractions were resistant.

3 A series of nine antagonists (the above and WB4101, benoxathian, phentolamine, BMY 7378, HV 723, spiperone) were investigated against contractions to noradrenaline. The correlation with the potency of the series of α_1-adrenoceptor antagonists against contractions to noradrenaline was significant only for the α_{1A}-adrenoceptor ligand binding site ($r=0.88$, $n=9$, $P<0.01$).

4 In epididymal portions (nifedipine 10 μM), the isometric contraction to a single electrical pulse is α_1-adrenoceptor mediated. The correlation with ligand binding sites for 11 antagonists (the above plus ARC 239 and (+)-niguldipine) was significant only for the α_{1D}-adrenoceptor subtype ($r=0.65$, $n=11$, $P<0.05$).

5 In conclusion, tonic contractions of rat vas deferens produced by exogenous agonists are mediated predominantly by α_{1A}-adrenoceptors, although a second subtype of receptor may additionally be involved in phasic contractions. Nerve-stimulation evoked α_1-adrenoceptor mediated contractions seem to predominantly involve non-α_{1A}-adrenoceptors, and the receptor involved resembles the α_{1D}-receptor.

Keywords: Rat vas deferens; α_{1A}-adrenoceptors; α_{1B}-adrenoceptors; α_{1D}-adrenoceptors; prazosin; RS17053; 5-methyl-urapidil; A61603

Abbreviation: CEC, chloroethylclonidine

Introduction

α_1-Adrenoceptors were initially subdivided into α_{1A}- and α_{1B}-subtypes in ligand binding studies, based on the affinities of a series of ligands, especially WB 4101 and prazosin (Morrow & Creese, 1986), and based on the ability of the alkylating agent chloroethylclonidine (CEC) to inactive the α_{1B}- but not the α_{1A}-subtype (Han *et al*., 1987). Molecular cloning techniques revealed initially four subtypes of α_1-adrenoceptor: α_{1a}, α_{1b}, α_{1c}, α_{1d} (Cotecchia *et al*., 1988; Schwinn *et al*., 1990; Lomasney *et al*., 1991; Perez *et al*., 1991; see Bylund *et al*., 1994). However, the α_{1a} and α_{1d} clones showed 99.8% homogeneity and appeared to represent the same subtype. It is now clear that the α_{1a}/α_{1d} clone represents a novel subtype of α_1-adrenoceptor (α_{1D}), whereas the α_{1c} is now identified with the α_{1A}-ligand binding site. These clones have now been renamed to match the functional receptors: α_{1A} (formely α_{1c}), α_{1B} (formerly α_{1b}), and α_{1D} (formerly α_{1a}/α_{1d}).

One of the earliest functional subclassifications of α_1-adrenoceptors was into α_{1H} and α_{1L}, with high and low affinity for prazosin (see Flavahan & Vanhoutte, 1986 but see Docherty, 1987; Muramatsu *et al*., 1990). α_1-Adrenoceptors in blood vessels were further subdivided into three subtypes, α_{1H}, α_{1N} and α_{1L}, based on their affinities for prazosin, WB 4101 and HV 723 (Muramatsu *et al*., 1990). Under this classification, α_{1H}-receptors have high affinity for prazosin, and appear to match the α_{1A}, α_{1B}, α_{1D} classes (Muramatsu *et al*., 1995), whereas α_{1L} and α_{1N} have low affinity for prazosin and do not seem to match current molecular cloning based classifications.

New α_1-adrenoceptor antagonists have been developed for effects in the lower urinary tract to treat benign prostatic hypertrophy, and the receptors involved were identified as α_{1A}-adrenoceptors. However, some antagonists which were selective for α_{1A}-adrenoceptors in ligand binding studies had low potencies in functional studies of the lower urinary tract (RS 17053: Ford *et al*., 1996; Kenny *et al*., 1996; SB 216469: Chess-Williams *et al*., 1996).

It has been variously suggested that contractions of rat vas deferens to exogenous noradrenaline or adrenaline are mediated predominantly by α_{1A}-adrenoceptors (Han *et al*., 1987; Hanft & Gross, 1989; Aboud *et al*., 1993), or α_{1L}- in addition to α_{1A}-adrenoceptors (Ohmura *et al*., 1992). The objects of this study were to re-examine the α_1-adrenoceptor subtypes present in rat vas deferens in the light of current knowledge of α_1-adrenoceptor subtypes and using some of the newer subtype selective compounds. Some of these results have been published in shortened form (Docherty, 1998).

Methods

Male Wistar rats (200–300 g) were obtained from Trinity College Dublin, and vas deferens was employed as outlined below.

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Rat vas deferens

Whole vas deferens, or prostatic or epididymal portions were obtained. Tissues were attached to myograph transducers under 1 g tension in organ baths at 37°C in Krebs–Henseleit solution of the following composition: (mM): NaCl 119; $NaHCO_3$ 25; D-glucose 11.1; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.0; EDTA 0.03, ascorbic acid 0.28. Additionally, cocaine (3 μM), propranolol (3 μM) and indomethacin (10 μM) were present except where otherwise stated.

In investigations of contractions produced by exogenous agonists, tissues were equilibrated for 30 min and then contracted with noradrenaline (10 μM). Bathing fluid was then changed every 15 min for the next hour. Following 60 min exposure to antagonist or vehicle, a single agonist concentration response-curve was obtained per tissue. Tissues were contracted with noradrenaline, methoxamine, phenylephrine or A61603 administered cumulative in 0.5 log unit increments beginning with 1 nM. Antagonist potency was expressed as the dissociation constant K_B from the equation $K_B = [B]/(DR-1)$, where [B] is the concentration of antagonist and DR is the agonist dose-ratio produced by the antagonist as compared to the vehicle experiment, or as a pA_2 Value. Antagonist pA_2 values were obtained from the x-intercept of the plot of log (agonist DR-1) against log antagonist concentration, where the slope was not significantly different from negative unity (Arunlakshana & Schild, 1959). Slopes of Schild plots not significantly different from -1.0 were constrained to -1.0, and a pK_B was obtained. In cases when slopes of Schild plots were significantly different from negative unity, or when a single concentration of antagonist was employed, antagonist potency was expressed as a pK_B from the effects of a single effective concentration of antagonist.

In experiments investigating the ability of competitive antagonists to inhibit the isometric twitch in epididymal portions of vas deferens (in the presence or absence of cocaine), tissues were placed between platinum electrodes and stimulated every 5 min with a single stimulus (0.5 ms pulses, supramaximal pulses) to produce isometric contractions, and nifedipine (10 μM) was present to block the non-noradrenergic component of the twitch. Antagonists or vehicle were added cumulative in 1 log unit increments at 5 min intervals. An isometric twitch was obtained following 5 min exposure to each antagonist concentration, or following exposure to the vehicle.

Drugs

A61603 (N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydro-naphthalen-1-yl]-methanesulfonamide; a gift from: Abbott Laboratories, U.S.A.); ARC239 (2-(2,4-(o-methoxyphenyl)-piperazin-1-yl)-ethyl-4,4 dimethyl-1,3-(2H,4H)-isoquinolindine chloride; a gift from: Karl Thomae, Germany); benoxathian hydrochloride (Research Biochemicals, U.S.A.); BMY 7378 (8-[2-(4-(2-methoxyphenyl) piperazin-1-yl)ethyl]-8-azaspiro[4,5]decane-7,9-dione; Research Biochemicals, U.S.A.); cocaine hydrochloride (Sigma, U.K.); corticosterone (Sigma, U.K.); HV723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)-amino)-propyl)-benzene acetonitrile fumarate; a gift from: Hokurika, Japan), methoxamine hydrochloride (Research Biochemicals, U.S.A.); 5-methyl-urapidil (a gift from: Byk, Germany); nifedipine (Sigma, U.K.); (+)-niguldipine (Sigma, U.K.); noradrenaline bitartrate (Sigma, U.K.); phentolamine hydrochloride (Research Biochemicals, U.S.A.); phenylephrine hydrochloride (Sigma, U.K.); prazosin hydrochloride (a gift from: Pfizer, Sandwich, U.K.); propranolol hydrochloride (Sigma, U.K.); RS17053 (N-[2(2-cyclopropylmethoxy)ethyl]-5-chloro-α, α-dimethyl-1H-indole-3-ethylamine hydrochloride; (a gift from: Roche Bioscience, U.S.A.); spiperone (Research Biochemicals, U.S.A.); WB 4101 (2-(2′,6′-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxan; Research Biochemicals, U.S.A.).

Drugs were dissolved in distilled water, except for corticosterone and spiperone (100% ethanol), (+)-niguldipine (100% methanol) and RS 17053 (DMSO).

Statistics

Values are mean ± s.e.mean from n experiments. Agonist pD_2 ($-\log EC_{50}$) values before and after vehicle or test antagonist were compared, and effects of antagonist on nerve stimulation-evoked responses were compared with the effects of vehicle, by Student's t-test for paired or unpaired data where appropriate, and by Analysis of Variance. Statistical and graphical analysis was carried out using Instat for Macintosh and GraphPad Prism for PC. Correlations were made between functional antagonist potency and pooled binding affinity data from a number of studies: there are limitations in using pooled data statistically as the aggregate mean may not have the required normal distribution.

Results

Whole vas deferens: responses to exogenous agonists

In whole vas deferens, agonists produced contractions consisting of intermittent spikes superimposed on a tonic contraction: phasic contractions were measured as the total increase above baseline and so includes the spike and tonic components (see Figure 1). The agonists noradrenaline, methoxamine, phenylephrine and A61603 produced tonic contractions with pD_2 values of 5.84 ± 0.12, 5.53 ± 0.07, 5.52 ± 0.06 and 7.98 ± 0.16 g and maximum contractions of 1.34 ± 0.09, 1.05 ± 0.08, 1.40 ± 0.07 and 1.33 ± 0.09 g, respectively ($n = 21$ in all cases). The maximum tonic contraction to methoxamine was significantly less than the maximum to the other agonists (Analysis of Variance, $P < 0.05$). The agonists noradrenaline, methoxamine, phenylephrine and A61603 produced phasic contractions with pD_2 values of 5.59 ± 0.11, 5.28 ± 0.10, 5.25 ± 0.08 and 7.74 ± 0.15 g and maximum contractions (measured as the maximum height of tonic contractions combined with intermittent spikes) of 2.11 ± 0.11, 2.04 ± 0.10, 2.26 ± 0.15 and 2.16 ± 0.11 g, respectively ($n = 21$ in all cases).

The effects of prazosin (10–30 nM), RS 17053 (100 nM) and 5-methyl-urapidil (30 nM) on tonic contractions to noradrena-

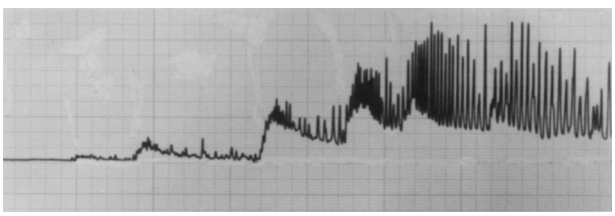

Figure 1 Tracing of a typical experiment illustrating the tonic and phasic nature of contractions to agonists in rat vas deferens. This experiment shows the effects of increasing concentrations of noradrenaline (0.1–30 μM). Tonic contractions were taken as the peak sustained contraction, and phasic contractions as the maximum intermittent tension (including sustained) obtained. Time scale: 20 min of recording shown.

line and phasic contractions to the four agonists are shown in Figures 2–3. Phasic contractions are omitted for the other three antagonists, since tonic and phasic contractions were affected similarly by antagonists. Prazosin produced parallel shifts in all cases, and all antagonists produced parallel shifts in the potency of A61603. RS 17053 and 5-methyl-urapidil tended to produce apparently non-parallel shifts in the potency of noradrenaline and methoxamine, and to a lesser extent phenylephrine, but the resistant component was small (Figures 2–3).

However, a component particularly of the phasic contractions were resistant to high concentrations of antagonists; this was particularly evident for RS 17053 against noradrenaline. The effects of high concentrations of RS 17053 were investigated against tonic and phasic contractions to noradrenaline in terms of absolute tension developed (Figure 4). RS 17053 (1 μM) significantly reduced the maximum tonic contraction to 0.57 ± 0.10 g ($n=4$), without affecting maximum phasic contractions. RS 17053 (10 μM) significantly reduced tonic contractions to 0.18 ± 0.05 g ($n=9$) and significantly reduced phasic contractions to 1.14 ± 0.22 g ($n=9$). In the figure, noradrenaline pD_2 for phasic contractions in vehicle experiments was 5.59 ± 0.21, and the pD_2 was shifted approximately 10 fold by RS 17053 (0.1 μM) (pD_2) of 4.50 ± 0.14) and by RS 17053 (1 μM) (pD_2 of 4.59 ± 0.26 g) (Figure 4). A noradrenaline pD_2 following RS 17053 (10 μM) could not be obtained due to a significant reduction in the maximum response (Figure 4). It is clear from the figure that, although low concentrations of RS 17053 (0.1 μM) significantly shifted the potency of noradrenaline, there was a phasic

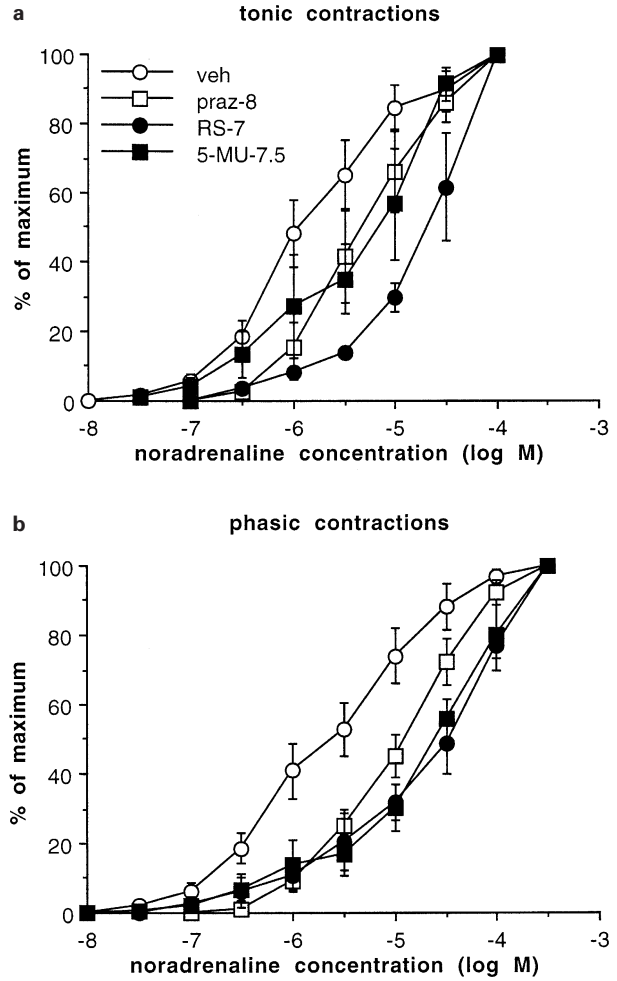

Figure 2 Tonic (a) and phasic (b) contractions obtained to noradrenaline in whole vas deferens, following exposure to vehicle (veh), prazosin (10 nM) (praz-8), RS 17053 (100 nM) (RS-7) or 5-methyl-urapidil (30 nM) (5-MU-7.5). Values are expressed as percentage of the maximum for each experiment. Vertical bars represent s.e.mean from at least three experiment. For clarity, vehicle experiments are combined but, since pK_B values were obtained in experiments with paired vehicles, the shift in agonist potency produced by antagonists is not readily obtained from these curves.

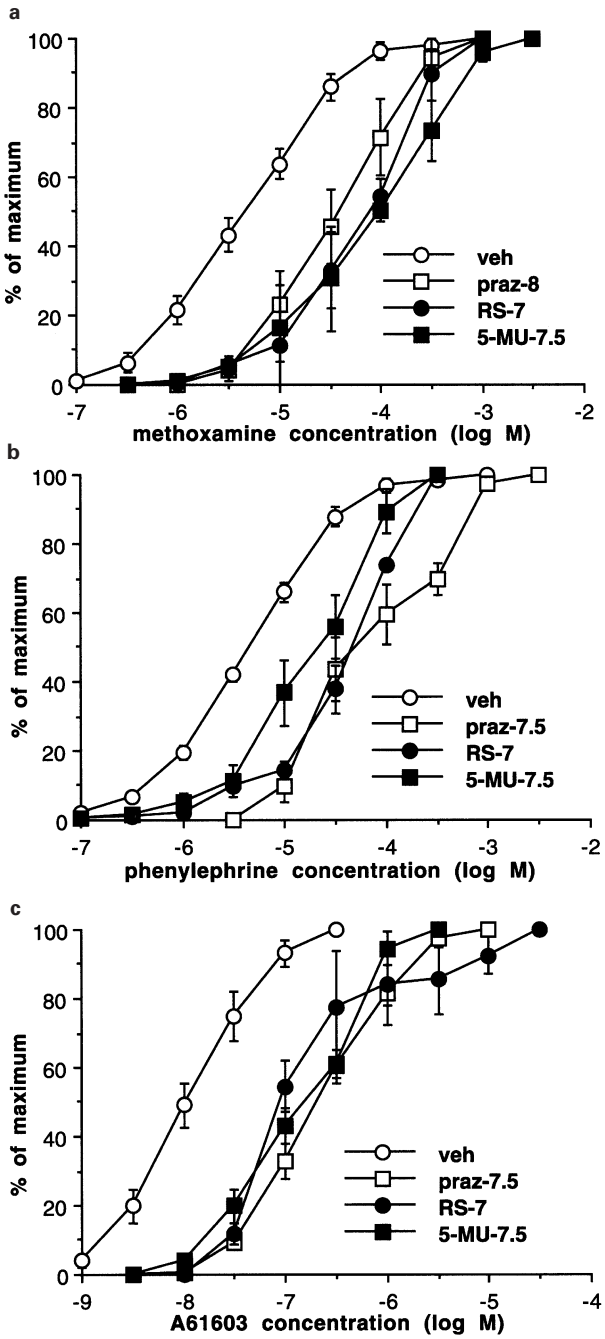

Figure 3 Phasic contractions obtained to methoxamine (a), phenylephrine (b) and A 6163 (c) in whole vas deferens, following exposure to vehicle (veh), prazosin (10 or 30 nM) (praz-8 or −7.5), RS 17053 (100 nM) (RS-7) or 5-methyl-urapidil (30 nM) (5-MU-7.5). Values are expressed as percentage of the maximum for each experiment. Vertical bars represent s.e.mean from at least three experiments. For clarity, vehicle experiments are combined but, since pK_B values were obtained in experiments with paired vehicles, the shift in agonist potency produced by antagonists is not readily obtained from these curves.

component of the response resistant even to RS 17053 (10 μM) (Figure 4).

Prazosin produced concentration-dependent shifts in the potency of all four agonists, and behaved competitively in that it did not decrease the maximum contraction, and slope of the Schild plot were not significantly different from negative unity, so that pA_2 values could be calculated (Table 1). However, in terms of slope of Schild plots, 5-methylurapidil behaved non-competitively against phenylephrine and RS17053 behaved competitively against methoxamine (see Table 1). However, pK_B values from the effects of the lowest effective concentration were calculated whenever pA_2 values could not be calculated (Table 1).

In addition to prazosin, 5-methyl-urapidil and RS 17053, WB4101, benoxathian, phentolamine, BMY 7378, HV 723 and spiperone were investigated against contractions to noradrenaline. pA_2 values were converted to pK_B values by constraining the slope of Schild plots to -1. The pK_B values obtained are shown in Table 2. For some of the antagonists, pK_B was calculated from the effects of a single concentration of antagonist producing an approximate 1 log unit (10 fold) shift

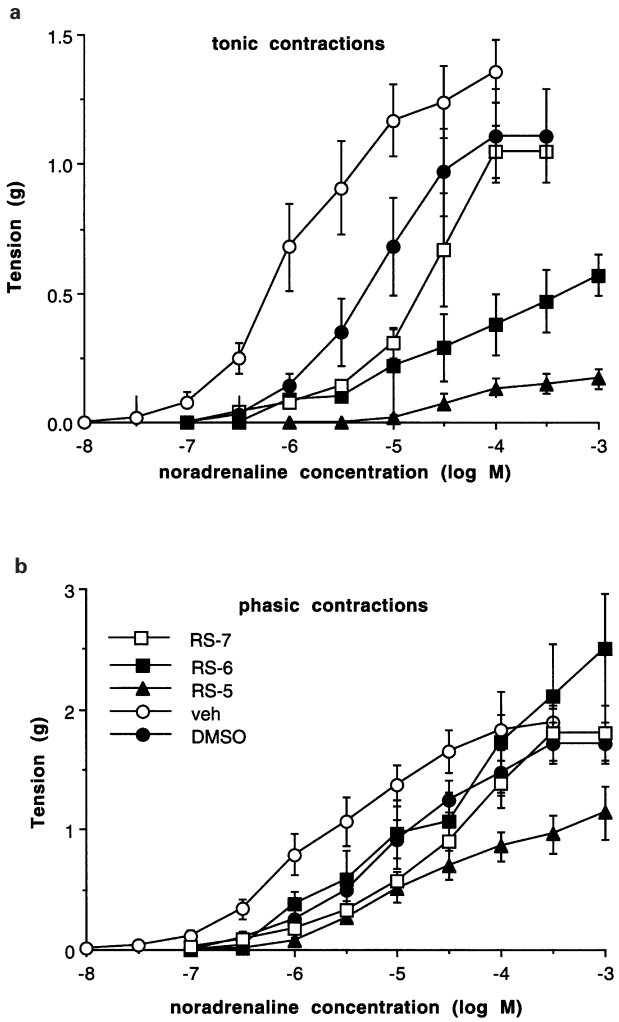

Figure 4 Tonic (a) and phasic (b) contractions obtained to noradrenaline in whole vas deferens expressed as absolute tension (g), following exposure to vehicle (veh), RS 17053 (100 nM) (RS-7), RS 17053 (1 μM) (RS-6), DMSO or RS 17053 (10 μM) (RS-5). Vertical bars represent s.e.mean from at least four experiments. For clarity, vehicle experiments for the two lower concentrations of RS 17053 are combined but, since DMSO, the vehicle for the highest concentration of RS 17053 (10 μM), also produced significant effects, this is included separately.

in agonist potency: (μM): RS 17053 0.1, 1.16 ± 0.06 g; phentolamine 1, 1.28 ± 0.25 g; BMY 7378 1, 0.48 ± 0.14 g; BMY 10, 1.42 ± 0.30 g; HV 723 0.1, 1.24 ± 0.10 g; spiperone 1, 1.16 ± 0.18 g. These potencies were correlated with affinities for α_{1A}, α_{1B} and α_{1D}-adrenoceptor ligand binding sites obtained by taking the mean of published values (see Tables 2, 3 and Figure 5). The correlation with the potency of the series of α_1-adrenoceptor antagonists against contractions to noradrenaline was significant for the α_{1A}-adrenoceptor ligand binding site ($r = 0.88$, $n = 9$, $P < 0.01$) (Figure 5). One of the published studies examined seven of the antagonists used in this study (all except benoxathian and spiperone; Ford *et al*., 1996). Correlation at antagonist potency with the ligand binding data of Ford *et al*. (1996) was again significant only for the α_{1A}-adrenoceptor (α_{1A} $r = 90$, $P < 0.01$; α_{1B} $r = 0.66$, n.s.; α_{1D} $r = 0.62$, $n = 9$, n.s.).

Epididymal portions of rat vas deferens: nerve-mediated responses

In epididymal portions of rat vas deferens, the initial biphasic twitch to a single electrical stimulus was reduced to a monophasic response by nifedipine (10 μM), which eliminates the first (non-noradrenergic) phase, leaving the second (noradrenergic) phase. Under these conditions, the twitch to a single stimulus was 0.80 ± 0.05 g ($n = 68$), and antagonists reduced the size of the twitch in a concentration-dependent manner (Figure 6). Antagonist potency was slightly reduced in the presence of cocaine, but the correlation between antagonist potency with and without cocaine was very close ($r = 0.98$) so that cocaine did not change relative potencies (Figure 7). A total of 11 antagonists were examined (those listed above plus ARC239 and (+)-niguldipine) and potencies (without cocaine) are shown in Table 2. These potencies were correlated with affinities for α_{1A}, α_{1B} and α_{1D}-adrenoceptor ligand binding sites obtained by taking the mean of published values (see Tables 2, 3 and Figure 8). The correlation with ligand binding sites was significant only for the α_{1D}-adrenoceptor subtype ($r = 0.65$,

Table 1 Potencies of antagonists against contractions to noradrenaline, methoxamine, phenylephrine and A61603 in rat vas deferens

	Prazosin	*5-methyl-urapidil*	*RS17053*
Noradrenaline			
pA_2	8.59 ± 0.18	8.33 ± 0.19	/
slope	-1.07 ± 0.23	-0.98 ± 0.19	0.53 ± 0.16
pK_B	8.64 ± 0.11	8.31 ± 0.09	8.16 ± 0.16
	($n = 19$)	($n = 9$)	($n = 5$)
Methoxamine			
pA_2	9.34 ± 0.33	8.92 ± 0.60	8.42 ± 0.40
slope	-0.93 ± 0.16	-1.06 ± 0.36	-0.92 ± 0.22
pK_B	9.21 ± 0.06	9.02 ± 0.16	8.30 ± 0.12
	($n = 9$)	($n = 6$)	($n = 10$)
Phenylephrine			
pA_2	8.62 ± 0.34	/	/
slope	-1.03 ± 0.28	-0.55 ± 0.09	-0.21 ± 0.06
pK_B	8.66 ± 0.10	8.10 ± 0.27	8.11 ± 0.03
	($n = 9$)	($n = 3$)	($n = 3$)
A61603			
pA_2	8.66 ± 0.21	8.48 ± 0.28	/
slope	-1.17 ± 0.21	-1.06 ± 0.28	-0.22 ± 0.05
pK_B	8.85 ± 0.09	8.54 ± 0.10	7.96 ± 0.20
	($n = 7$)	($n = 10$)	($n = 4$)

Potencies are expressed as pA_2 values from Schild plots with slope ($\pm$s.e.mean), followed by a pK_B obtained by constraining the slope to -1, or as a pK_B ($\pm$s.e.mean) from the effects of a single concentration of antagonist. Number of experiments are indicated by *n*.

Table 2 Potencies of antagonists against contractions to noradrenaline in rat vas deferens

		pA₂	*slope*	*pKB*	*pIC₅₀*
1.	Prazosin	8.59±0.11 (19)	−1.07±0.23	8.64±0.19	7.36±0.15 (4)
2.	5-methyl-urapidil	8.33±0.19 (9)	−0.98±0.19	8.31±0.09	8.97±0.29 (9)
3.	RS 17053			8.16±0.06 (5)	<5.00 (5)
4.	Benoxathian	9.02±0.27 (9)*	−0.98±0.19	8.99±0.10	7.46±0.08 (5)
5.	WB 4101	9.54±0.33 (6)*	−0.94±0.28	9.47±0.10	8.13±0.12 (4)
6.	HV 723			8.24±0.10 (3)	7.79±0.36 (5)
7.	BMY 7378			6.48±0.14 (5)	7.45±0.04 (5)
8.	Phentolamine			7.28±0.25 (5)	5.23±0.11 (4)
9.	Spiperone			7.16±0.18 (4)	5.88±0.19 (3)
10.	ARC 239				8.22±0.18 (4)
11.	(+)-niguldipine				<5.00 (4)

*Values taken from Aboud *et al.* (1993). Potencies are expressed as pA_2 values from Schild plots with slope (±s.e.mean), followed by a pK_B obtained by constraining the slope to −1, or as a pK_B (±s.e.mean) from the effects of a single concentration of antagonist. Number of experiments are indicated by *n*. Also shown are pIC_{50} values obtained against nerve-stimulation-evoked contractions in epididymal portions of rat vas deferens (in the absence of cocaine). Values in parentheses are number of experiments. Compounds are numbered to match numbers used in Figures 5 and 8.

Table 3 Published antagonists affinities for α_{1A}-, α_{1B}- and α_{1D}-ligand binding sites

	α1A	*α1B*	*α1D*
1. Pazosin (*n*=8)	9.41±0.18	9.66±0.17	9.63±0.08
2. 5-methyl-urapidil (*n*=7)	8.85±0.15	7.09±0.14	7.69±0.20
3. RS 17053 (*n*=2)	8.85±0.25	7.55±0.25	7.45±0.35
4. Benoxathian (*n*=3)	8.96±0.18	8.15±0.42	8.46±0.13
5. WB 4101 (*n*=7)	9.63±0.20	8.31±0.22	9.17±0.18
6. HV 723 (*n*=1)	9.40	9.70	8.50
7. BMY 7378 (*n*=2)	6.32±0.22	6.74±0.50	8.78±0.62
8. Phentolamine (*n*=6)	8.44±0.30	7.52±0.29	7.64±0.28
9. Spiperone (*n*=3)	7.81±0.44	8.60±0.33	7.98±0.23
10. ARC 239 (*n*=1)	9.34	9.02	9.42
11. (+)-niguldipine (*n*=5)	8.81±0.48	7.15±0.37	6.88±0.25

Data from Perez *et al.* (1991); Schwinn & Lomasney (1992); Fature *et al.* (1994); Forray *et al.* (1994); Goetz *et al.* (1995); Knepper *et al.* (1995); Marshall *et al.* (1996); Ford *et al.* (1996); Kenny *et al.* (1996); Buckner *et al.* (1996); Chess-Williams *et al.* (1996). Values are mean and s.e.mean, with the *n* the number of different published values. Compounds are numbers to match numbers used in Figures 5 and 8.

$n=11$, $P<0.05$) (Figure 8). One of the published studies examined eight of the antagonists used in this study (all except ARC 239, benoxathian and spiperone; Ford *et al.*, 1996). Correlation of antagonist potency with the ligand binding data of Ford *et al.* (1996) was not significant for any α_1-adrenoceptor subtype (α_{1A} $r=0.01$, n.s.; α_{1B} $r=0.16$, n.s.; α_{1D} $r=0.53$, n.s.).

Discussion

In this study, we have looked at subtypes of α_1-adrenoceptor mediating contractions of the rat vas deferens to exogenous agonists (noradrenaline, methoxamine phenylephrine or A61603) and contractions to endogenous noradrenaline. These will be discussed separately, beginning with contractions to exogenous agonists.

Responses to exogenous agonists

In whole vas deferens, the agonists noradrenaline, methoxamine, phenylephrine and A61603 produced concentration-dependent contractions which consisted of intermittent spikes superimposed on a smaller tonic contraction: all agonists behaved apparently similarly in terms of producing tonic and phasic contractions. Phasic contractions were measured as the total contraction, i.e. the tonic contraction plus the intermittant spike. Agonists tended to be slightly more potent at producing tonic than phasic contractions, but this was simply because the tonic contraction dominated at low agonist concentrations (see Figure 1) and the maximum phasic contraction was significantly greater than the maximum tonic contraction. The potencies of noradrenaline, phenylephrine and A61603 obtained in this study are similar to potencies obtained to these agonists in rat vas deferens by Knepper *et al.* (1995). In the absence of antagonists, both tonic and phasic contractions to all agonists showed monophasic concentration-response curves.

The antagonist prazosin produced approximately parallel shifts in the potency of all four agonists, and RS 17053 and 5-methyl-urapidil produced parallel shifts in the potency of A61603. However, for the agonists noradrenaline and methoxamine (and to a lesser extent phenylephrine), both RS 17053 and 5-methyl-urapidil tended to produce non-parallel shifts such that the potency of the agonist was shifted more at higher than lower concentrations. However, these differences were relatively small and difficult to quantify.

We chose to compare antagonist potencies against noradrenaline with published ligand binding data for the three α_1-adrenoceptor sites; we followed the method of Marshall *et al.* (1996) in taking the mean of several published ligand binding affinities for each antagonist at each site (see Table 3). We chose nine antagonists; RS 17053, BMY 7378, phentolamine, HV723 and spiperone in addition to the above: BMY 7378 is especially interesting as an α_{1D}-selective antagonist (Goetz *et al.*, 1995) which has higher potency as an antagonist in rat aorta, but lower potency in vas deferens (Deng *et al.*, 1996). Given the evidence for two components to the contractile response, it is perhaps not surprising that some Schild plots to RS 17053 and 5-methyl-urapidil demonstrated non-competitive interactions. The correlation between published data for ligand binding sites and the antagonist potencies obtained in our results would confirm the identification of the predominant receptor mediating contractions of rat vas deferens to noradrenaline as an α_{1A}-adrenoceptor. This agrees with other studies of agonist contractions in rat vas deferens (Teng *et al.*, 1994; Burt *et al.*, 1995). However, it is clear from the above that a second receptor is involved in contractions to noradrenaline, but it appears that none of the antagonists employed is selective for that receptor, although 5-

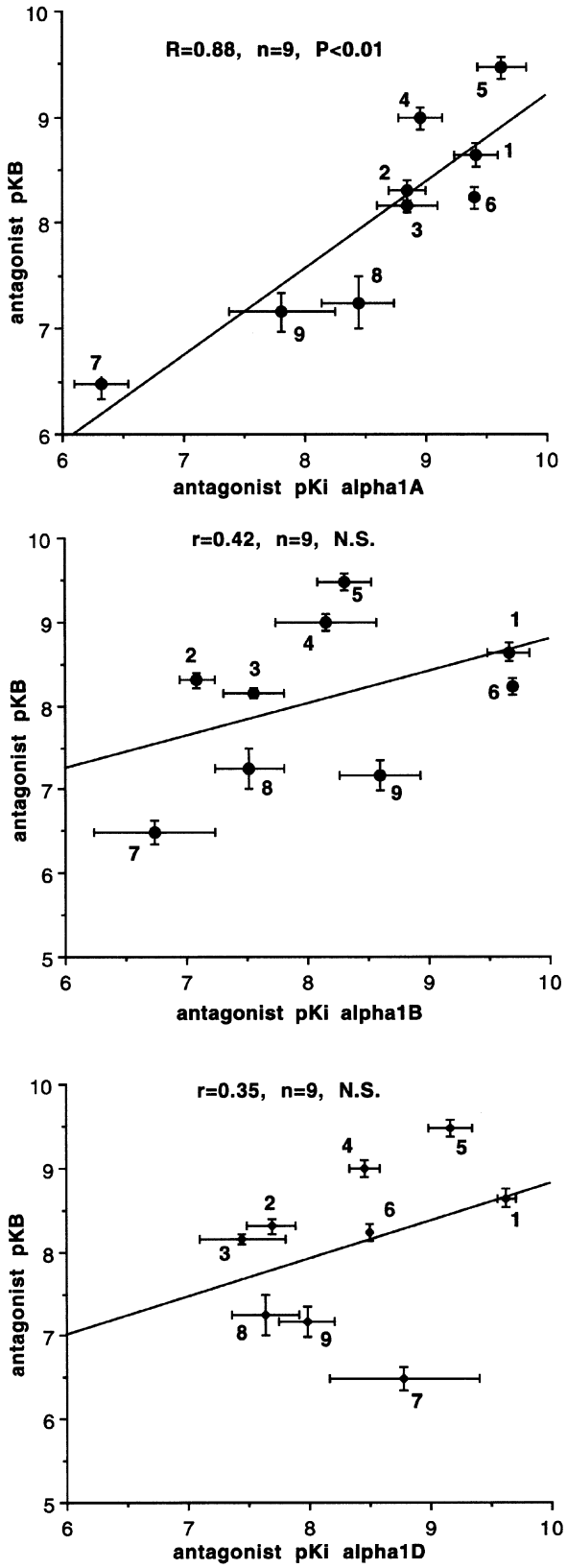

Figure 5 Correlation between published antagonist affinity for α_{1A}-, α_{1B}- and α_{1D}-ligand binding sites (data from Perez *et al.*, 1991; Schwinn & Lomasney, 1992; Faure *et al.*, 1994; Forray *et al.*, 1994; Goetz *et al.*, 1995; Knepper *et al.*, 1995; Marshall *et al.*, 1996; Ford *et al.*, 1996; Kenny *et al.*, 1996) and antagonist postjunctional potency (pK_B) against contractions to noradrenaline in whole vas deferens. Numbers indicate compounds as listed in Tables 2 and 3. Vertical and horizontal bars indicate s.e.mean. There was a significant correlation between antagonist postjunctional potency and affinity for α_{1A}-adrenoceptor ligand binding site.

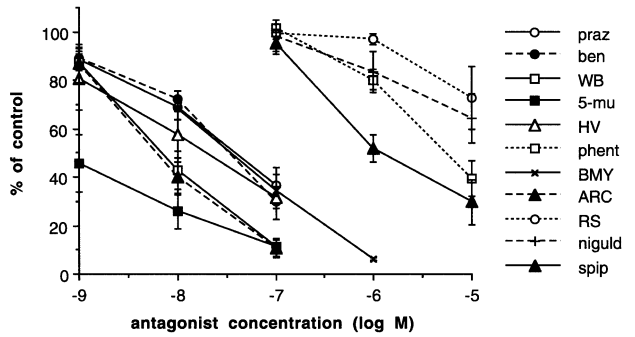

Figure 6 Effects of antagonists on the contractile response of epididymal portions of rat vas deferens in the presence of nifedipine (10 μM) to eliminate non-adrenergic contractions: prazosin (praz), benoxathian (ben), WB 4101 (WB), 5-methyl-urapidil (5-mu), HV 723 (HV), phentolamine (phen), BMY 7378 (BM). ARC 239 (ARC), RS 17053 (RS), (+)-niguldipine (niguld), spiperone (spip). Vertical bars represent s.e.mean from at least three experiments.

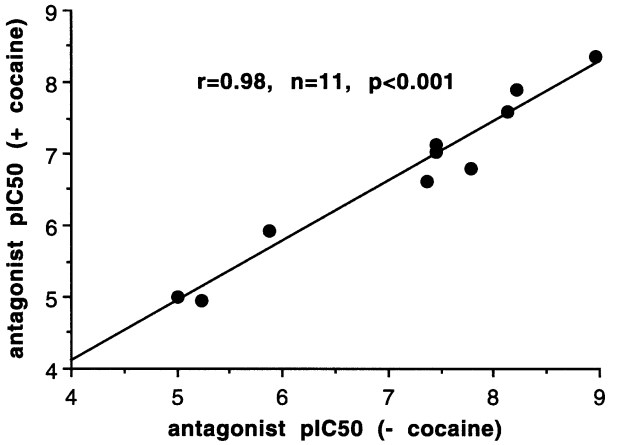

Figure 7 Correlation between antagonist postjunctional potency (pIC_{50}) against stimulation-evoked contractions to a single stimulus in epididymal portions of rat vas deferens in the presence of nifedipine (10 μM) with and without cocaine. There was a very significant correlation between potencies with and without cocaine.

methyl-urapidil and RS 17053 showed low potency at this second site. A component of the response to noradrenaline in rat vas deferens resistant to 5-methyl-urapidil has previously been reported in studies in which the antagonist was given subsequent to the agonist (Bultmann *et al.*, 1994).

High concentration of RS 17053 virtually abolished tonic contractions to noradrenaline, presumably by competitive antagonism, but were less effective against phasic contractions. However, the problem in comparing phasic concentrations with and without high concentrations of antagonist is the phasic contractions have a large tonic component in the absence of antagonist which may obscure the spikes. Both tonic and phasic contractions were abolished by pretreatment with phenoxybenzamine (10 μM), and the alkylating agent CEC (100 μM) did not significantly affect tonic or phasic contractions to noradrenaline, but directly produced phasic contractions (spikes) (Aboud *et al.*, 1993, and unpublished). In the presence of the calcium entry blocker nifedipine, the intermittent spikes produced by noradrenaline were abolished, and a small tonic contraction remained (Aboud *et al.*, 1993). Since the phasic contraction to noradrenaline could not be

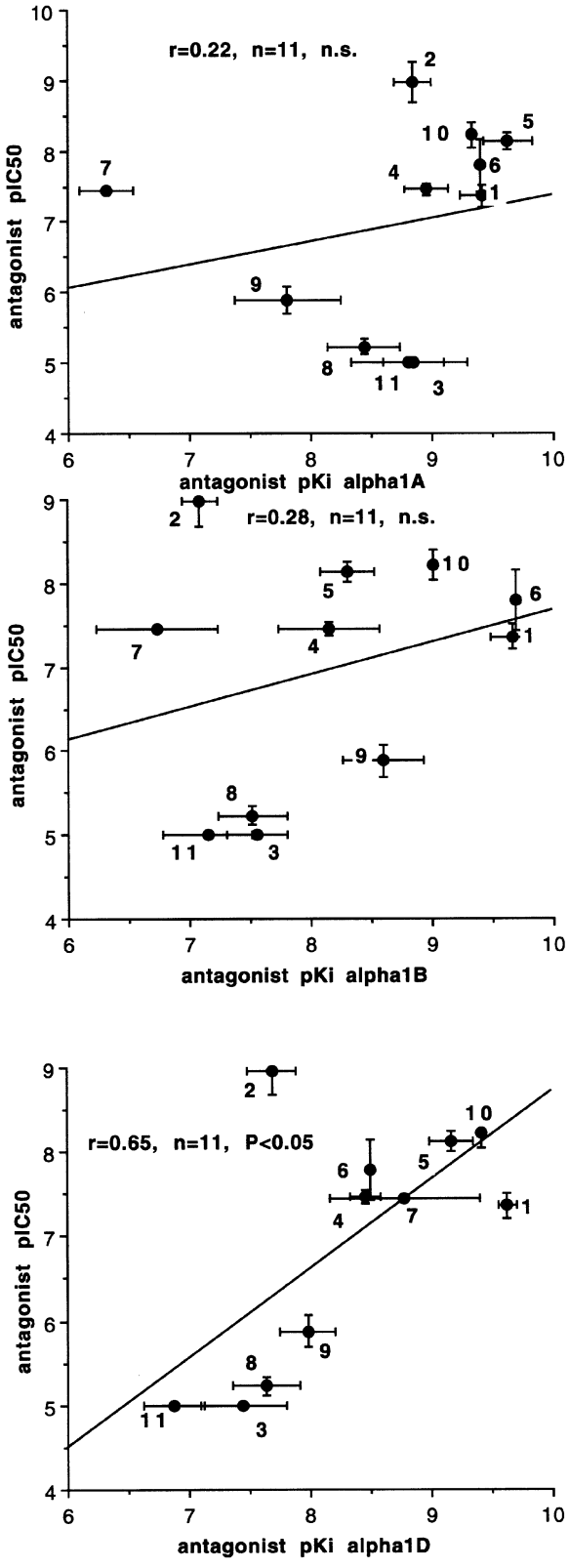

Figure 8 Correlation between published antagonist affinity for α_{1A}-, α_{1B}- and α_{1D}-ligand binding sites (data from Perez *et al*., 1991; Schwinn & Lomasney, 1992; Faure *et al*., 1994; Forray *et al*., 1994; Goetz *et al*., 1995; Knepper *et al*., 1995; Marshall *et al*., 1996; Ford *et al*., 1996; Kenny *et al*., 1996) and antagonist postjunctional potency (pIC_{50}) against stimulation-evoked contractions to a single stimulus in epididymal portions of rat vas deferens in the presence of nifedipine (10 μM). Numbers indicate compounds as listed in Tables 2 and 3. Vertical and horizontal bars indicate s.e.mean. There was a significant correlation between antagonist potency and affinity for the α_{1D}-adrenoceptor ligand binding site.

examined in isolation, the α_1-adrenoceptor mediated response to nerve stimulation was examined in more detail.

Responses to endogenous noradrenaline

In rat whole vas deferens, the electrical stimulation-evoked contraction to a single stimulus consists of a biphasic response, the first phase of which is non-adrenoceptor mediated and predominates in prostatic portions, and the second phase of which is α_1-adrenoceptor mediated, and predominates in the epididymal portion (Brown *et al*., 1983). The second α-noradrenergic phase can be examined in isolation in the epididymal portion in the presence of nifedipine which eliminates the second non-noradrenergic response. Antagonist potencies at inhibiting this stimulation-evoked α_1-adrenoceptor mediated contraction were obtained both in the absence and presence of cocaine. Cocaine blocks the uptake of noradrenaline back into the nerve terminal, and so prolongs and potentiates contractions: antagonists tended to be less potent at inhibiting contractions in the presence of cocaine, but antagonist potencies with or without cocaine correlated well with each other. Antagonist potencies at inhibiting nerve-evoked contractions in epididymal portions did not correlate with α_{1A}-adrenoceptors, but significantly correlated only with the α_{1D}-adrenoceptor ligand binding site, even though the correlation was relatively low ($r=0.65$), due largely to one outlier, 5-methyl-urapidil. Interestingly, when 5-methyl-urapidil is eliminated from the correlation, there was a much improved correlation with α_{1D}-adrenoceptors ($r=0.90$, $P<0.001$). In the present study, the relatively high potency of BMY 7378 against nerve stimulation-evoked contractions (pIC_{50} of 7.45) may also suggest an α_{1D}-adrenoceptor. Since the α_{1D}-receptor is reported to be expressed in rat vas deferens (Perez *et al*., 1991; Rokosh *et al*., 1994), the above receptor may represent a function α_{1D}-adrenoceptor. Bultmann *et al*. (1994) investigated nerve mediated responses in rat vas deferens and found that 5-methyl-urapidil was approximately ten times less potent than WB 4101, a potency which would also aid identification with α_{1D}-adrenoceptors. The high potency of 5-methyl-urapidil in our study is difficult to explain, but may suggest other actions of this compound. Because of this, we re-examined the potency of 5-methyl-urapidil: in our experiments the pIC_{50} varied between 10.0 and 8.00, with a mean of 8.97. This range of values is much greater than for other antagonists (e.g. benoxathian: 7.22–7.70). Although 5-methyl-urapidil is reported to bind with high affinity to 5-HT-1A receptors (Zilles *et al*., 1991), its inhibitory action in rat vas deferens is not antagonized by the 5-HT-1 receptor antagonist cyanopindolol (authors, unpublished). An α_{1D}-adrenoceptor should show some sensitivity to block by CEC, and the nerve-evoked adrenergic contraction of rat vas deferens is indeed blocked by CEC (Mallard *et al*., 1992; Aboud *et al*., 1993), but this action of CEC is at least partly prejunctional (Bultmann & Starke, 1993). An α_{1D}-adrenoceptor has also been reported to mediated nerve-evoked pressor responses in the pithed rat preparation (Castillo *et al*., 1998).

Finally, how do we explain the contradictory findings in the literature concerning the α_1-adrenoceptor subtype mediating contractions of rat vas deferens? Clearly with two subtypes present, different experimental conditions may favour the dominance of one or other of the subtypes so that both α_{1A}- and α_{1L}-adrenoceptors have been reported. Genetic polymorphism of α_{1A}-adrenoceptors does not explain α_{1L}-adrenoceptors, since all variants have been found to have similar pharmacological characteristics (Shibata *et al*., 1996). The α_{1A}-adrenoceptor, expressed in the CHO-K1 cell line, displayed

binding properties of the α_{1A}-adrenoceptor but functionally, in terms of inositol phosphate accumulation, the receptor displayed properties of the α_{1L}-adrenoceptor (Ford *et al*., 1996). Hence, presumably due to factors determined by the methodology employed, and perhaps due to environmental differences around the receptor, the same receptor can show characteristics of both α_{1A}- and α_{1L}-adrenoceptors: hence α_{1A}- and α_{1L} may be different affinity/conformational states of the α_{1A}-adrenoceptor. The additional presence of an α_{1D}-adrenoceptor complicates matters further.

In conclusion, we have studied contractile responses of rat vas deferens to exogenous agonists and to electrical stimulation. Tonic and phasic contractions of rat vas deferens produced by exogenous agonists are mediated predominantly by α_{1A}-adrenoceptors, although particularly phasic contractions may have a second component. Nerve-evoked contractions seem to be mediated predominated by non-α_{1A}-adrenoceptors, and the receptor involved resembles the α_{1D}-receptor.

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