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α_1-Adrenoceptor-induced contractility in rat aorta is mediated by the α_{1D} subtype

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Abstract

Adrenoceptor agonists were used to characterize the α_1-adrenoceptor subtype responsible for mediating tension (phasic and tonic combined) in the denuded rat aorta and compared with radioligand binding at α_1-adrenoceptor subtypes. The rank order of potency at the rat aorta was the same as that obtained for binding affinity at the rat clonal α_{1d}-adrenoceptor: norepinephrine > epinephrine > cirazoline > phenylephrine > oxymetazoline > A-61603 > methoxamine. Correlation coefficients comparing rat aortic contraction (pD_2) to binding (pK_i) were 0.09–0.21 for $\alpha_{1A/a}$ receptors, 0.66 for clonal α_{1b} and 0.94 for clonal α_{1d}-adrenoceptors. Correlation coefficients comparing the clonal α_{1d}-adrenoceptor binding affinity to in vitro contractile responses were 0.03 and 0.10 for the rat vas deferens and canine prostate α_{1A}-adrenoceptor responses, respectively, 0.09 for the rat spleen α_{1B} and as noted, 0.94 for the rat aorta. The agreement observed between agonist potency at the rat aorta and affinity for the α_{1d} binding site provide new evidence that the α_{1D}-adrenoceptor subtype is responsible for mediating contractions in the rat aorta.

Keywords: α_{1D}-Adrenoceptor; Radioligand binding; Contractility; Aorta, rat

1. Introduction

Pharmacological characterization of the α_1-adrenoceptor responsible for mediating contraction in the rat aorta has been limited to the use of the α_{1A}-adrenoceptor selective antagonist 5-methyl-urapidil (Gross et al., 1989), the α_{1B}-adrenoceptor alkylating agent chloroethylclonidine, the Ca^{2+} channel blocking agent nifedipine (Minneman, 1988) and antagonists that possess inadequate selectivity for the α_{1D}-adrenoceptor subtype (Testa et al., 1995). Tension generated by α_1-adrenoceptor agents in the rat aorta has been described as mediated by both the α_{1A}- and α_{1B}-adrenoceptor (Tian et al., 1990; Piascik et al., 1991), α_{1B}-adrenoceptor (Han et al., 1990; Testa et al., 1995) or atypical (Muramatsu et al., 1991). Recently, Kenny et al. (1995), presented evidence showing that the contractile response to norepinephrine is primarily mediated by the α_{1D}-adrenoceptor. He used 11 antagonists possessing a wide range of radioligand binding affinities and selectivities for the three α_1-adrenoceptor subtypes. We arrived at a similar conclusion using a completely different approach. In our studies we compared the radioligand binding affinity (pK_i) to in vitro functional potency (pD_2) of standard agonists. These agonists, like the antagonists selected by Kenny et al. (1995), possessed sufficient differences in functional potencies and radioligand binding affinities to construct persuasive regression correlations. This approach also avoided the agonist-antagonist interactions that potentially complicate the analysis when non-competitive blockade occurs (Aboud et al., 1993; Kenny et al., 1995).

Receptors used for α_1-adrenoceptor binding assays included rat submaxillary gland α_{1A} (Michel et al., 1989), bovine clonal α_{1a}, human clonal α_{1a}, hamster clonal α_{1b} and rat clonal α_{1d} (Knepper et al., 1995). The α_1-adrenoceptor functional models included the rat vas deferens α_{1A} (Aboud et al., 1993), canine prostate α_{1A} (Kenny et al., 1994), rat spleen α_{1B} (Aboud et al., 1993), and the rat aorta α_{1D} (Guarino et al., 1995). In this work current nomenclature for α-adrenoceptors is as described by Bylund et al. (1994) and Ford et al. (1994) whereby clonal receptors have lower case subscripts while tissue derived receptor material is referred to with capitalized subscripts and the nomenclature follows that recommended by IUPHAR (Bylund et al., 1994).

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2. Materials and methods

2.1. Functional models

Male Sprague Dawley rats (200–350 g) were sedated with CO_2 and decapitated. The thoracic aorta, entire vas deferens and spleen were removed and immediately placed into Krebs Ringer bicarbonate solution with the following mM concentrations: 120 NaCl, 20 $NaHCO_3$, 11 dextrose, 4.7 KCl, 2.5 $CaCl_2$, 1.5 $MgSO_4$, 1.2 KH_2PO_4, 0.01 K_2EDTA, equilibrated with 5% CO_2-95% O_2 (pH 7.4 at 37°C). Propranolol 0.004 mM was included in all of the assays to block β-adrenoceptors. The endothelium was removed from the aorta by passing a 100 mm length of PE-160 tubing through the lumen and the aortic tissue cut into 3–4 mm rings and mounted in 10 ml tissue baths. One end was fixed to a stationary glass rod and the other to a Grass FT03 transducer at a basal preload of 1.0 g. Tissues were allowed to equilibrate for at least 90 min and primed with 10 μM phenylephrine. Absence of functional endothelium in the rat aorta was confirmed by loss of the acetylcholine-induced relaxation response at the peak of the phenylephrine prime response. A control concentration response curve was generated for each tissue using PE as the control agonist. After a 90 min rinse period a second response curve was generated for the test agonist. The amount of agent necessary to cause a 50% response (ED_{50}) was calculated using 'ALLFIT' (DeLean et al., 1980) and agonist potencies indexed to phenylephrine and expressed as the negative logarithm (pD_2). Each tissue was used for only one test agonist. For the antagonists, the test agent was allowed 30 min exposure time before a second phenylephrine curve was generated. The potency, expressed as a pA_2, was calculated according to the method of Arunlakshana and Schild (1959). The individual tissues were exposed to only one concentration of the test antagonist. The regression lines of the Schild plots were analyzed using least squares regression (Snedecor and Cochran, 1980). The remaining isolated tissues were pharmacologically treated in a similar manner. The entire vas deferens was desheathed and spirally cut from the prostatic end as far as possible into the epididymal portion and mounted in tissue baths with a resting tension of 0.5 g. The spleen was split longitudinally into two preparations per rat and mounted as above at a resting tension of 1.0 g. Only dogs that were greater than 2 years of age were used for the canine prostate assays. The animals were sacrificed by means of an intravenous injection of pentobarbital solution, Somlethal, J.A. Webster, Sterling, MA, USA. The entire prostate was removed and sliced parallel to the plane of the urethra. Excess glandular material was removed so as to obtain a 4–5 mm strip that contained a small amount of glandular tissue attached to the smooth muscle capsule. The prostate was preloaded to 0.5 g tension. Agonist concentration response curves were cumulative except for the vas deferens assay in which the transient response made such measurements impractical.

2.2. Radioligand binding assays

Receptor binding assays were performed essentially as described previously (Knepper et al., 1995). Briefly, the cDNA encoding α_1 receptors was obtained from TULCO (Triangle Universities Licensing Consortium, Research Triangle Park, NC, USA) and expressed in mouse fibroblast cells (LTK^-) grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 30 μM geneticin. Roller bottle cultures of cloned cell lines were used to provide cell membranes for receptor characterization studies. Cells at confluence were washed twice with phosphate-buffered saline (PBS, Sigma, 120 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4-NaH_2PO_4, pH 7.4) and detached from the roller bottles by incubating for 15 min at 37°C in a Tris-EDTA solution (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4). Cells were washed in PBS with centrifugation at $3000 \times g$ for 5 min at 2–4°C, resuspended, recentrifuged, washed in 40 volumes 5 mM Tris-HCl, 5 mM EDTA, pH 7.7, and centrifuged at $40000 \times g$ for 10 min. Cells were homogenized in 10 ml of 50 mM Tris-HCl, 5 mM EDTA (pH 7.4) and homogenates centrifuged at $35000 \times g$ for 10 min. The pellets were rehomogenized in 50 mM Tris-HCl (pH 7.4) and centrifuged as before. The homogenates were resuspended in 6.25 volumes (per gram wet weight) of 50 mM Tris-HCl and aliquots frozen in $N_2(l)$ and stored at −70°C until the time of assay. Rat submaxillary glands were also used as a source of α_{1A} receptors and were prepared as described (Michel et al., 1989). Receptor binding assays were performed using the method of Greengrass and Bremner (1979) as further described by Giardina et al. (1993) in 1 ml final volumes containing 500 μl of tissue homogenate diluted in 50 mM Tris-HCl to yield 0.834 mg wet weight for the cloned receptors and 3.33 mg wet weight for the submaxillary gland tissue, 450 μl of [^{3}H]-prazosin (0.2 nM, final concentration) and 50 μl of either water (total binding), 10 μM phentolamine (non-specific binding) or competing compounds at various concentrations. Data were analyzed as previously described (Hancock et al., 1988).

2.3. Drugs and chemicals

The drugs used were: (±)-propranolol hydrochloride, *l*-phenylephrine hydrochloride, (−)-norepinephrine bitartrate, (−)-epinephrine bitartrate, prazosin hydrochloride, methyl sulfoxide (Sigma Chemical Co., St. Louis, MO); 5-methyl-urapidil and chloroethylclonidine dihydrochloride (Research Biochemicals International, Natick, MA, USA); terazosin hydrochloride, WB4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride), cirazoline hydrochloride, oxymetazoline hydrochloride, methoxamine hydrochloride, alfuzosin hydro-

Table 1
Functional potency and efficacy of agonists at the rat vas deferens α_{1A}-, canine prostate α_{1A}-, rat spleen α_{1B}- and denuded rat aorta α_{1D}-adrenoceptor

	Rat vas deferens α_{1A}	Canine prostate α_{1A}	Rat spleen α_{1B}	Rat aorta α_{1D}
Norepinephrine	5.93 (0.08)	5.66 (0.15)	4.99 (0.05)	7.92 (0.11)
	[1.81] 105%	[1.08] 117%	[1.22] 215%	[5.02] 130%
Epinephrine	6.54 (0.05)	6.30 (0.11)	5.23 (0.13)	7.80 (0.13)
	[4.14] 109%	[3.82] 88%	[0.75] 288%	[5.50] 115%
Cirazoline	7.14 (0.06)	7.00 (0.15)	4.02 (0.18)	7.46 (0.05)
	[18.3] 93%	[15.8] 135%	[0.11] 109%	[1.05] 107%
Phenylephrine	5.78 (0.10)	5.53 (0.08)	4.96 (0.12)	6.90 (0.03)
	[1.43] 97%	[0.74] 116%	[1.03] 102%	[0.66] 111%
Oxymetazoline	6.54 (0.21)	6.46 (0.16)	6.46 (0.39)	5.77 (0.21)
	[4.99] 80%	[11.2] 71%	[27.4] 35%	[0.04] 71%
A-61603	8.25 (0.07)	7.74 (0.11)	6.50 (0.10)	5.59 (0.07)
	[458] 109%	[222] 91%	[14.2] 90%	[0.02] 100%
Methoxamine	5.46 (0.09)	5.59 (0.07)	3.97 (0.08)	5.54 (0.13)
	[0.39] 84%	[0.38] 114%	[0.05] 97%	[0.03] 105%

Data expressed as $pD_2 \pm$ (S.E.M.), [phenylephrine index], % of maximum control phenylephrine response, $n \geq 4$.

chloride, phentolamine hydrochloride, doxazosin hydrochloride, indoramin hydrochloride, HEAT (2-[β-(4-hydroxyphenyl)-ethylaminomethyl]tetralone hydrochloride), ARC239 ([2-[2-[4-(*o*-methoxyphenyl)piperazine-1-yl]-ethyl]-4,4-dimethyl-1,3(2*H*-4*H*)isoquinolinedione]dihydrochloride), A-61603 (*N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methanesulfonamide hydrobromide) (Abbott Laboratories, North Chicago, IL, USA).

3. Results

Phenylephrine caused isometric contractions (phasic and tonic combined) of the denuded rat aorta with a p*D*2 of 6.90 ± 0.03 (Table 1, Fig. 1) and a maximum contraction of 71 ± 1.93 cg. The pD_2 of test agonists was derived from the second curve (test response) and compared to the first curve (phenylephrine control response). An index was calculated based upon relative agonist potencies compared to phenylephrine in individual tissues for a given day. In the denuded rat aorta oxymetazoline caused a 71% maximum response compared to phenylephrine. All of the other agonists tested produced maximum responses equal to or greater than phenylephrine (Table 1). The relative potencies indexed to phenylephrine ranged from 0.02 for A-61603, an α_{1A} subtype selective agonist (Knepper et al., 1995), to 5.5 for epinephrine (Table 1). Cirazoline was potent in all assays except for the rat spleen α_{1B} model. The rat spleen exhibited the most variability in maximum response to the various agonists with oxymetazoline (35%) causing the least response and epinephrine (288%) the greatest response compared to phenylephrine (Table 1). Methoxamine was consistently the weakest agonist tested in each of the four functional models.

The rank order of agonist potency (pD_2) in the rat aorta was: norepinephrine (7.92) > epinephrine (7.80) > cirazoline (7.46) > phenylephrine (6.90) > oxymetazoline (5.77) > A-61603 (5.59) ≥ methoxamine (5.54) (Table 1). This same rank order was seen in the binding affinity (pK_i) at the rat clonal α_{1d}-adrenoceptor: norepinephrine (7.69) > epinephrine (7.45) > cirazoline (7.32) > phenylephrine (6.79) > oxymetazoline (6.47) > A-61603 (5.87) > methoxamine (5.24) (Table 2). Linear coefficients of correlation (r), were used to compare the various α_1-adrenoceptor subtype selective functional models to the

Table 2
Radioligand binding affinity for agonists at the rat submaxillary α_{1A}-, bovine clonal α_{1a}-, human clonal α_{1a}-, hamster clonal α_{1b}- and rat clonal α_{1d}-adrenoceptor

	Rat submax. α_{1A}	Bovine clonal α_{1a}	Human clonal α_{1a}	Hamster clonal α_{1b}	Rat clonal α_{1d}
Norepinephrine	6.61 (0.02)	6.36 (0.02)	6.38 (0.03)	6.54 (0.02)	7.69 (0.03)
Epinephrine	6.97 (0.07)	6.62 (0.08)	6.64 (0.06)	6.91 (0.06)	7.45 (0.07)
Cirazoline	7.44 (0.15)	7.02 (0.09)	7.00 (0.03)	6.70 (0.09)	7.32 (0.07)
Phenylephrine	6.01 (0.11)	6.03 (0.26)	5.76 (0.06)	5.90 (0.09)	6.79 (0.08)
Oxymetazoline	8.19 (0.08)	7.88 (0.14)	7.86 (0.11)	6.69 (0.06)	6.47 (0.15)
A-61603	8.05 (0.08)	7.52 (0.03)	7.61 (0.05)	5.68 (0.19)	5.87 (0.08)
Methoxamine	5.25 (0.25)	5.26 (0.26)	5.54 (0.06)	4.35 (0.16)	5.24 (0.22)

Data shown as $pK_i \pm$ (S.E.M.) $n \geq 4$.

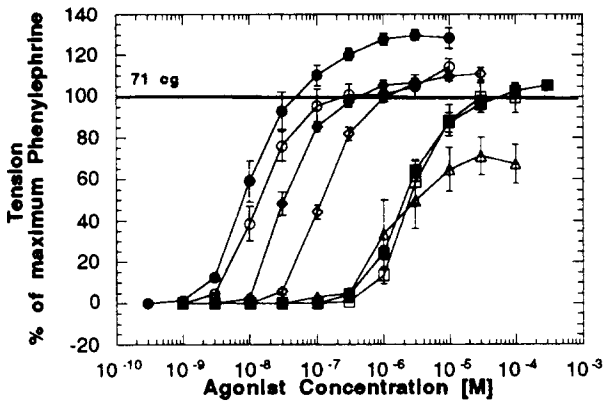

Fig. 1. Tension as a percent of maximum phenylephrine response ± S.E.M. in the rat aorta. Norepinephrine, filled circles; epinephrine, open circles; cirazoline, filled diamonds; phenylephrine, open diamonds; methoxamine, filled squares; A-61603, open squares; oxymetazoline, open triangles, $n \geq 4$ for each agonist.

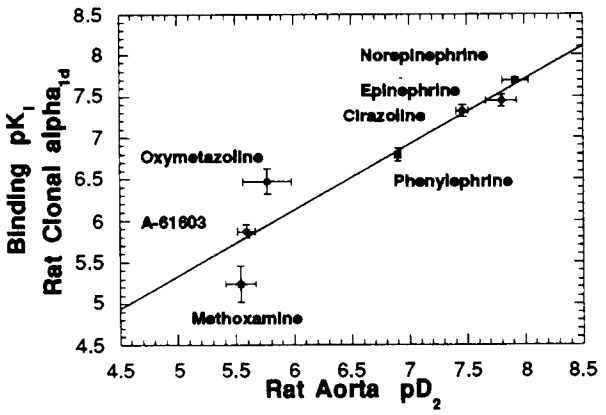

Fig. 2. Comparison of functional potency ($pD_2 \pm$S.E.M.) to radioligand binding affinity ($pK_i \pm$S.E.M.) in the rat aorta, $n \geq 4$ for each agonist.

α_1-adrenoceptor selective radioligand binding assays. Plotting the rat aorta pD_2 on the abscissa and the binding affinity pK_i (α_{1d}-cloned) on the ordinate produced a coefficient of correlation $r = 0.939$ and a slope = 0.792 (Table 3, Fig. 2). None of the other functional models demonstrated this strong correlation between agonist potency and α_{1d} receptor binding affinity: rat vas deferens (0.028), canine prostate (0.104), and rat spleen (0.094), (Table 3). Except for the rat clonal α_{1d} receptor, none of the other α-adrenoceptor subtype binding assays demonstrated agreement between agonist potency and radioligand binding affinity: rat submaxillary α_{1A} (0.089), bovine clonal α_{1a} (0.128), human clonal α_{1a} (0.208) and hamster clonal α_{1b} (0.663) (Table 3).

The contractile response of the denuded rat aorta was sensitive to the Ca^{2+} entry blocker nifedipine showing a concentration-dependent shift to the right of the phenylephrine response curve with a reduction in maximum response (Fig. 3). This preparation was also sensitive to the alkylating effects of chloroethylclonidine resulting in a shift of the phenylephrine response curve to the right with a reduction in the maximum response to phenylephrine. The effective concentration for inactivating the chloroethylclonidine-sensitive component in the rat aorta was 30 μM for 30 min, followed by a 4 h rinse period Fig. 4).

The antagonists examined were competitive in nature, with Schild plot slopes not different from unity and with no reduction in maximum responses (Table 4). The current antagonists did not show a range of subtype selectivity comparable to the agonists. The most selective agent in any of the functional models was doxazosin, showing an 83-fold selectivity for the rat spleen α_{1B} vs. the canine prostate α_{1A}-adrenoceptor. 5-Methyl-urapidil was 39 times more potent in the canine prostate α_{1A}- than the rat spleen α_{1B}-adrenoceptor model. Prazosin was 36-fold selective for the rat spleen α_{1B}- vs. the canine α_{1A}-adrenoceptor

Table 3
A comparison of functional potency (pD_2) to radioligand binding affinity (pK_i) for the standard agonists

		Rat vas deferens α_{1A}	Canine prostate α_{1A}	Rat spleen α_{1B}	Rat aorta α_{1D}
Rat submaxillary	α_{1A}	0.809	0.806	0.730	0.089
		0.941 *	0.902 *	0.621 *	0.144 *
Bovine clonal	α_{1a}	0.758	0.757	0.759	0.128
		0.938 *	0.892 *	0.639 *	0.143 *
Human clonal	α_{1a}	0.782	0.798	0.745	0.208
		0.964 *	0.940 *	0.620 *	0.032 *
Hamster clonal	α_{1b}	0.263	0.230	0.294	0.663
		0.286 *	0.219 *	0.170 *	0.878 *
Rat clonal	α_{1d}	0.028	0.104	0.094	0.939
		0.027 *	0.097 *	0.040 *	0.980 *

Data represent the correlation coefficients obtained by linear regression for each of the α_1-adrenoceptor subtype selective assays (* results with oxymetazoline omitted).

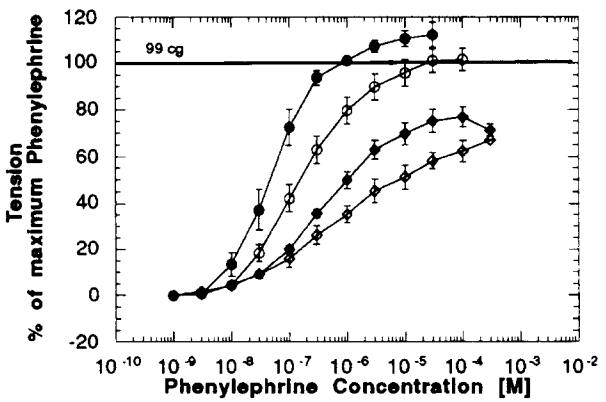

Fig. 3. Effects of nifedipine on contraction caused by phenylephrine in the denuded rat aorta. Vehicle, filled circles; 10 nM nifedipine, open circles; 1.0 μM nifedipine, closed diamonds; 100 μM, open diamonds. Each point is the mean ± S.E.M. of three determinations.

Table 4
Functional potency of standard antagonists at the rat vas deferens α_{1A}, canine prostate α_{1A}, rat spleen α_{1B} and the denuded rat aorta α_{1D} models

	Rat vas deferens α_{1A}	Canine prostate α_{1A}	Rat spleen α_{1B}	Rat aorta α_{1D}
Tamsulosin	9.41 (0.27)	9.39 (0.21)	9.69 (0.44)	10.60 (0.43)
	1.22 (0.28)	1.26 (0.13)	0.84 (0.15)	0.94 (0.11)
	0.84	0.95	0.84	0.95
ARC239	8.47 (0.24)	8.98 (0.27)	8.86 (0.77)	9.46 (0.19)
	1.22 (0.14)	1.21 (0.16)	0.83 (0.35)	1.06 (0.05)
	0.96	0.95	0.83	0.99
WB4101	9.69 (0.40)	8.87 (0.34)	9.39 (0.26)	9.36 (0.30)
	0.86 (0.12)	1.12 (0.12)	0.91 (0.08)	1.15 (0.12)
	0.91	0.96	0.93	0.97
Prazosin	8.78 (0.30)	8.46 (0.15)	10.02 (0.16)	9.35 (0.20)
	1.17 (0.15)	0.97 (0.07)	1.07 (0.15)	1.18 (0.09)
	0.91	0.97	0.91	0.98
Doxazosin	8.69 (0.70)	7.59 (0.20)	9.51 (0.41)	8.97 (0.23)
	0.86 (0.21)	0.99 (0.18)	1.08 (0.21)	1.17 (0.13)
	0.70	0.91	0.90	0.95
Heat	8.60 (0.21)	8.45 (0.14)	8.80 (0.24)	8.86 (0.13)
	0.92 (0.25)	1.09 (0.11)	1.06 (0.08)	1.03 (0.06)
	0.84	0.96	0.97	0.99
Terazosin	8.04 (0.45)	7.44 (0.24)	8.60 (0.46)	8.65 (0.29)
	0.83 (0.17)	0.79 (0.09)	0.94 (0.14)	0.99 (0.13)
	0.84	0.85	0.91	0.95
Alfuzosin	7.61 (0.13)	6.66 (0.10)	8.31 (0.12)	8.41 (0.18)
	0.86 (0.07)	0.99 (0.04)	0.88 (0.05)	1.22 (0.09)
	0.98	0.98	0.98	0.96
5-Methyl-urapidil	8.39 (0.14)	8.76 (0.30)	7.17 (0.10)	7.83 (0.12)
	1.00 (0.09)	0.94 (0.12)	1.10 (0.06)	1.04 (0.07)
	0.96	0.94	0.95	0.98
Phentolamine	8.01 (0.07)	7.53 (0.07)	7.47 (0.27)	7.72 (0.16)
	0.92 (0.07)	1.08 (0.07)	0.81 (0.17)	1.23 (0.11)
	0.97	0.97	0.83	0.97
Indoramin	8.24 (0.71)	8.54 (0.40)	7.88 (0.41)	7.40 (0.44)
	0.78 (0.15)	1.13 (0.22)	1.11 (0.19)	1.10 (0.16)
	0.82	0.78	0.86	0.96

Data expressed as pA_2 (±S.E.M.), slope (±S.E.M.) and the coefficient of linear regression for the Schild plot, $n \geq 8$ tissues.

model. All of the other comparisons for antagonists showed a maximum of less than 16-fold selectivity for any particular α-adrenoceptor subtype (Table 4). For the rat aorta model, the range of subtype selectivity ranged from 3- to 24-fold with doxazosin showing the maximum separation comparing the rat aorta to the canine prostate model. This

Table 5
Radioligand binding affinities of standard antagonists at the rat submaxillary α_{1A}-, bovine clonal α_{1a}-, human clonal α_{1a}-, hamster clonal α_{1d}- and rat clonal α_{1d}-adrenoceptor

	Rat submax. α_{1A}	Bovine clonal α_{1a}	Human clonal α_{1a}	Hamster clonal α_{1b}	Rat clonal α_{1d}
Tamsulosin	10.55 (0.07)	10.51 (0.04)	10.54 (0.04)	9.69 (0.05)	10.17 (0.04)
ARC239	9.34 (0.12)	9.78 (0.13)	9.41 (0.03)	9.02 (0.11)	9.42 (0.12)
WB4101	10.01 (0.23)	10.36 (0.13)	10.0 (0.04)	9.20 (0.12)	9.92 (0.12)
Prazosin	9.95 (0.12)	9.71 (0.07)	9.65 (0.07)	10.26 (0.07)	10.0 (0.07)
Doxazosin	9.37 (0.23)	9.04 (0.11)	9.10 (0.07)	9.64 (0.13)	9.30 (0.18)
HEAT	9.58 (0.10)	9.74 (0.06)	9.44 (0.04)	9.68 (0.11)	9.27 (0.03)
Terazosin	9.08 (0.13)	8.48 (0.07)	8.69 (0.06)	9.16 (0.06)	8.96 (0.07)
Alfuzosin	8.62 (0.12)	8.19 (0.11)	7.52 (0.18)	9.06 (0.06)	8.75 (0.10)
5-Methyl-urapidil	8.82 (0.10)	9.20 (0.07)	8.56 (0.06)	7.42 (0.04)	7.86 (0.05)
Phentolamine	8.43 (0.06)	8.85 (0.06)	8.55 (0.01)	8.14 (0.07)	8.28 (0.06)
Indoramin	8.35 (0.07)	8.84 (0.16)	8.55 (0.06)	8.32 (0.14)	7.36 (0.10)

Data shown as pK_i ± (S.E.M.), $n \geq 4$.

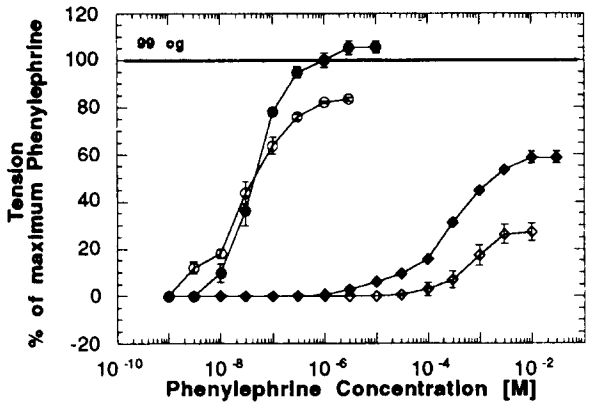

Fig. 4. Effects of chloroethylclonidine on contraction caused by phenylephrine in the denuded rat aorta. The tissues were exposed to chloroethylclonidine for 30 min and rinsed for 4 h before the second phenylephrine curve was generated. Vehicle, filled circles; 3.0 μM chloroethylclonidine, open circles; 30 μM chloroethylclonidine, filled diamonds; 300 μM chloroethylclonidine open diamonds. Each point is the mean ± S.E.M. of three determinations.

same lack of subtype selectivity for the antagonists was seen with the radioligand binding assays (Table 5). The most selective agent in the binding assays was 5 methylurapidil, displaying a 63-fold preference for the bovine clonal α_{1a} vs. the hamster clonal α_{1b}-adrenoceptor subtype. Comparing the α_1-adrenoceptor subtype affinities to the functional pA_2's demonstrated the weakness in using non selective antagonists for the analysis. The coefficients of correlation displayed a limited range (from 0.737 to 0.933, Table 6).

4. Discussion

This study employed α-adrenoceptor agonists to characterize the α_1-adrenoceptor responsible for contraction in the denuded rat aorta. In this assay, the agonists differed sufficiently in their subtype selectivities to make an identification of receptor subtypes possible. A direct comparison of the potency of the seven agonists in producing tension in the denuded rat aorta to their radioligand binding affinity at the rat clonal α_{1d}-adrenoceptor showed excellent agreement between the two assays. In contrast there was poor agreement when the potency in the rat aorta was compared to α_1-adrenoceptor binding affinity in the rat submaxillary α_{1A}, the bovine clonal α_{1a}, the human clonal α_{1a} and the rat clonal α_{1b} assay.

Comparing the binding affinity (pK_i) of the rat clonal α_{1d} to the potency (pD_2) in producing tension in the α_1-adrenoceptor functional models (the rat vas deferens α_{1A}, the canine prostate α_{1A}, the rat spleen α_{1B},) agreement was seen only between the rat aorta and the cloned α_{1d} site. Note that with the omission of oxymetazoline from data obtained in the rat vas deferens and canine prostate α_{1A}-adrenoceptor, the agreement between agonist potency and binding affinity for the remaining agents is significantly improved. Oxymetazoline presented a weaker potency in these functional models than the radioligand binding would have predicted. This is peculiar to oxymetazoline alone and may reflect some ancillary pharmacology of this agonist yet to be defined.

Aboud et al. (1993) concluded that the α_1-adrenoceptor mediating contraction in the rat aorta is neither α_{1A} nor α_{1B}. They further suggested that it may represent the functional α_{1D} site first described by Perez et al. (1991). In a recent publication by Testa et al. (1995) the α_1-adrenoceptor responsible for contraction in the rat aorta was characterized as α_{1B}. This conclusion was based partly on the use of what the author regarded as 'no absolutely subtype-selective antagonists' (Testa et al., 1995). Since the antagonists tested in this study show minimal subtype selectivity at the α_1-adrenoceptor binding sites and minimal functional subtype selectivity, no definitive conclusions can be made about the α_1-adrenoceptor subtype mediating contraction in the rat aorta using these particular antagonists. By including additional subtype selective α_1-adrenoceptor antagonists, Kenny et al. (1995) found evidence supporting the classification of the rat aortic α_1-adrenoceptor as belonging to the α_{1D} subtype. The inclusion of BMY 7378 as a potent and selective α_{1d} agent (compared to the α_{1a} and α_{1b} subtypes) and SNAP 1069 as a weak α_{1d} agent (compared to the α_{1a} and α_{1b} sites) enhanced the correlation between potency in the rat aorta and radioligand binding affinity for the cloned human α_{1d}-adrenoceptor. However, Kenny et al. (1995), found non-competitive interactions between norepinephrine and 8 of the 11 antagonists tested, including BMY 7378. Saussy et al. (1994) found BMY 7378 to competitively antagonize phenylephrine induced contractions in the rat aorta. A similar problem was seen by Aboud et al. (1993), using norepinephrine against the antagonists prazosin, WB4101, and benoxathian in the isolated rat spleen. When phenylephrine was substituted for norepinephrine contractions were inhibited in a competitive manner. In our laboratory using phenylephrine as the agonist, we were able to demonstrate competitive antagonism in each of the

Table 6
A comparison of the functional potency (pA_2) of standard antagonists to the radioligand binding affinity (pK_i)

		Rat vas deferens α_{1A}	Canine prostate α_{1A}	Rat spleen α_{1B}	Rat aorta α_{1D}
Rat submaxillary	α_{1A}	0.852	0.589	0.849	0.933
Bovine clonal	α_{1a}	0.920	0.851	0.570	0.737
Human clonal	α_{1a}	0.905	0.707	0.782	0.895
Hamster clonal	α_{1b}	0.410	0.053	0.936	0.739
Rat clonal	α_{1d}	0.667	0.310	0.898	0.919

Data represent the correlation coefficients using linear regression for each of the α_1-adrenoceptor subtype selective assays.

four functional models examined. However, the correlation coefficients comparing the in-vitro potency of these antagonists in the rat aorta to the binding affinities for the α_1-adrenoceptor subtypes were not definitive.

Testa et al. (1995) also showed the rat aorta to be sensitive to the α_{1B}-adrenoceptor alkylating effects of chloroethylclonidine and insensitive to the Ca^{2+} channel inhibitor nicardipine (endothelium intact), suggesting the presence of the α_{1B}-adrenoceptor subtype (Minneman, 1988). Our data show nifedipine to have a significant effect on the phenylephrine response in the denuded rat aorta, shifting the response curve for phenylephrine to the right and reducing the maximum response. These divergent responses to Ca^{2+} entry blockade may be the result of the absence or presence of endothelial cells (Auch-Schwelk and Vanhoutte, 1991). Our data strongly suggest that the lack of agreement between the α_{1b}-adrenoceptor binding affinity and the rat aorta pD_2 ($r = 0.663$) using the above agonists excludes the α_{1B}-adrenoceptor subtype as responsible for the contraction in the rat aorta, whereas the coefficient of correlation for the α_{1d} and the rat aorta was 0.939. These same comparisons using antagonists demonstrate the weakness of antagonist based analysis due to the lack of subtype selectivity of most currently available agents which could result in the erroneous conclusion that the contraction in the rat aorta could be caused by any of the three α_1-adrenoceptor subtypes.

Although the availability of subtype selective antagonists is limited, several investigators have described two new agents that discriminate between the various subtypes. The selectivity of BMY 7378 for the α_{1d}-adrenoceptor has been shown by both radioligand binding assays and functional contractile models (Goetz et al., 1994; Saussy et al., 1994; Guarino et al., 1995). Discretamine is another recently described α_1-adrenoceptor antagonist that is reported to be selective for the α_{1D}-adrenoceptor. This agent shows selectivity for the α_{1D} over α_{1A} of 25-fold and α_{1D} over α_{1B} of 14-fold using the rat aorta, the rat vas deferens and the rat spleen functional tissues (Ko et al., 1994).

In summary, phenylephrine-induced contractions in the denuded rat aorta were sensitive to nifedipine, 5-methyl-urapidil and chloroethylclonidine, weakening the argument for the involvement of the α_{1A}- and the α_{1B}-adrenoceptor subtypes. The recent introduction of the α_1 subtype selective antagonists BMY 7378, (Goetz et al., 1994; Saussy et al., 1994; Guarino et al., 1995; Kenny et al., 1995), discretamine (Ko et al., 1994), and SNAP 1069 (Kenny et al., 1995) provide support for the α_1-adrenoceptor agonist-induced tension development in rat aorta being mediated by the α_{1D}-adrenoceptor. By virtue of the *competitive* blockade obtained in the current studies with antagonists, we also provide additional evidence for the pharmacological importance of the α_{1D}-adrenoceptor in the rat aorta. Our data showing a strong agreement between the potency of standard α-adrenoceptor agonists in α_1-adrenoceptor subtype selective functional models and radioligand binding affinity in α_1-adrenoceptor subtype selective assays provides novel evidence that the α_{1D}-adrenoceptor is responsible for α_1-agonist-induced contractile responses in the rat aorta.

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