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Characterization of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline

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1 The affinities of a number of α_1-adrenoceptor antagonists were determined by displacement of [^{3}H]-prazosin binding from cloned human α_{1A}-adrenoceptors (previously designated cloned α_{1c} subtype), α_{1B} α_{1D} and rat α_{1D}-adrenoceptors, stably expressed in rat-1 fibroblasts. Functional affinity estimates for these compounds were also determined from noradrenaline-mediated contractions of rat aorta.

2 BMY 7378 displayed high affinity for cloned human α_{1D}-adrenoceptors ($pK_i = 8.2 \pm 0.10$) and was selective over α_{1A} ($pK_i = 6.2 \pm 0.10$) and α_{1B} subtypes (6.7 ± 0.11). WB 4101, benoxathian and phentolamine displayed high affinity for α_{1A} and α_{1D} adrenoceptors compared to the α_{1B} subtype. Spiperone displayed high affinity and selectivity for α_{1B} adrenoceptors (pK_i 8.8 ± 0.16). 5-Methyl-urapidil was selective for cloned α_{1A} adrenoceptors.

3 Comparative binding affinities (pK_i) for compounds at cloned human and rat$_{1D}$ adrenoceptors were almost identical ($r = 0.99$, slope $= 1.08$).

4 Prazosin, doxazosin and 5-methyl-urapidil were potent, competitive antagonists of noradrenaline-mediated contractions of rat aorta (pA_2 values of 9.8, 8.8 and 7.8 respectively). The selective α_{1D} antagonist BMY 7378 was also a potent antagonist on rat aorta ($pK_B = 8.3 \pm 0.1$) but the interaction of this compound was not consistent with competitive antagonism at a single population of receptors.

5 Functional affinities for compounds determined against noradrenaline-mediated contractions of rat aorta correlated well with binding affinities at cloned α_{1D}-adrenoceptors ($r = 0.96$), but not with α_{1A} ($r = 0.61$) or α_{1B} ($r = 0.46$) subtypes.

6 Noradrenaline-mediated contractions of rat aorta were sensitive to the alkylating effects of chlorethylclonidine (CEC). CEC (10 μM) caused a small rightward shift in the noradrenaline concentration-response curve. CEC at 100 μM caused a further shift and suppression of the maximum response to noradrenaline.

7 The results of this study suggest that noradrenaline predominantly, but not exclusively, mediates contraction of rat aorta through the activation of an α_{1D}-adrenoceptor.

Keywords: α_1-Adrenoceptors; [^{3}H]-prazosin binding; rat aorta; adrenoceptor antagonists

Introduction

Pharmacological studies have consistently demonstrated the existence of at least two different α_1-adrenoceptor subtypes, α_{1A} and α_{1B}, in a number of tissues. Molecular cloning studies have done much to support the existence of multiple α_1 subtypes and three cloned subtypes have been identified and designated $\alpha_{1a/d}$, α_{1b} and α_{1c} (Bylund *et al.*, 1994). However, whilst the properties of cloned and tissue α_{1B} adrenoceptors are clearly similar, the relationship between cloned $\alpha_{1a/d}$ and α_{1c} subtypes in relation to endogenous receptors has only recently become clear (Ford *et al.*, 1994). When appropropriately defined in tissues such as rat submaxillary gland (Michel *et al.*, 1989) the pharmacological properties of tissue α_{1A}-adrenoceptors have been shown to be consistent with the cloned α_{1c} subtype (Faure *et al.*, 1994). Therefore, to remove ambiguity, both the cloned and endogenous subtype are referred to as an α_{1A} adrenoceptor (Bylund *et al.*, 1995). Thus, current α_1 adrenoceptor classification recognises three native and cloned subtypes which have been designated α_{1A}, α_{1B} and α_{1D} (used throughout this paper), corresponding to cloned subtypes previously designated as α_{1c}, α_{1b} and $\alpha_{1a/d}$ (Hieble *et al.*, 1995). Tissue correlates of cloned α_{1A} adrenoceptors have been demonstrated in rat submaxillary gland, human prostate and rabbit liver (Taddei *et al.*, 1993; Faure *et al.*, 1994) and correlates of the cloned α_{1B} subtype have been shown in rat liver and spleen (Kenny *et al.*, 1994a). However, whilst the presence of α_{1D}-adrenoceptors has been suggested in heterogeneous tissue populations (Michel & Insel, 1994), unequivocal demonstration of this endogenous subtype has not been shown to date. This in part has been precluded by the lack of selective compounds for this subtype, although recent preliminary evidence has indicated that BMY 7378 may be selective for cloned α_{1D}-adrenoceptors over other α_1 subtypes (Saussy *et al.*, 1994).

In an attempt to characterize the α_1-adrenoceptor mediating the contractile response of the rat aorta, several studies have concluded that the receptor cannot be classified as either α_{1A} or α_{1B} (Oriowo & Ruffolo, 1992; Aboud *et al.*, 1994) although others have suggested that a number of different subtypes contribute to the contractile response (Piascik *et al.*, 1991; van der Graaf *et al.*, 1993). Rat aorta expresses α_{1D}-adrenoceptors at the mRNA level (Perez *et al.*, 1991) and Aboud *et al.* (1994) suggested that since the pharmacological profile of the response could not be reconciled with either an α_{1A} or α_{1B} subtype, this tissue may possess a functional α_{1D}-adrenoceptor. In the present study we have characterized the pharmacological properties of rat aorta in comparison to cloned α_1-adrenoceptor subtypes and our data suggest that the contractile response of the rat aorta to noradrenaline is predominantly, but not exclusively, mediated by an α_{1D}-adrenoceptor.

A preliminary account of some these data has been presented to the British Pharmacological Society (Kenny *et al.*, 1994b; 1995).

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Methods

Radioligand binding studies of cloned α_1 adrenoceptors

Cloning of rat and human α_1 adrenoceptor cDNAs and stable transfection into rat-1 fibroblasts was carried out as previously described (Schwinn *et al.*, 1995). The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum and 300 μg ml^{-1} G418 sulphate, a neomycin analogue. For subculturing, cell monolayers were washed with Hank's balanced salt solution and trypsinized briefly with 0.05% trypsin, 0.5 mM EDTA and divided 1 : 5 to 1 : 20 every 3–4 days. Radioligand binding experiments were performed with membranes prepared from rat 1 fibroblast cells expressing individual α_1 subtypes. Scraped cells were homogenized in ice cold 50 mM Tris buffer (pH 7.5) with a Polytron homogenizer (PT10, setting 6, 20 s). The membranes were washed three times by centrifugation (20 min at 48, 000 *g*) and resuspended in fresh buffer before storage at −70°C. Cell membranes were stored at 0.5 mg ml^{-1} protein. Binding of [^{3}H]-prazosin was measured in 400 μl aliquots of diluted membranes (2–10 μg fibroblast protein) in 50 mM Tris buffer (pH 7.5) in a final assay volume of 500 μl. Non specific binding was determined in the presence of 1 μM phentolamine. Saturation isotherms were constructed by sequential dilution from a top concentration of 8 nM [^{3}H]-prazosin. Assays were incubated at 25°C with [^{3}H]-prazosin for 30 min and terminated by the addition of ice cold Tris buffer and rapid cold filtration over Whatman GF/B filters under vacuum. Saturation and displacement binding data were analysed by iterative non-linear curve fitting programmes (Graph PAD software, San Diego, U.S.A.).

Contractile responses of the rat aorta

Rings of thoracic aorta (approx 3–5 mm in length) from male Sprague-Dawley rats (250–300 g) were denuded of endothelium by gentle rubbing and suspended in organ baths under a resting tension of 1 g in Krebs Ringer bicarbonate of the following composition (mM); NaCl 120, KCl 5.5, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 20, glucose 11 and EDTA 0.03 and gassed with 95% O_2/5% CO_2. The solution also contained 10 μM cocaine, 10 μM corticosterone, 1 μM propranolol and 0.5 μM yohimbine. Tissues were exposed to a sensitizing dose of (−)-noradrenaline (100 μM) and washed over a 30 min period. Isometric contractions were obtained in response to cumulative additions of (−)-noradrenaline in the absence and presence of antagonists (incubated for 30 min). Antagonist pA_2 values were obtained from a plot of log (agonist DR-1) against log antagonist concentration where the slope was not different from unity (Arunlakshana & Schild, 1959). In other experiments, estimates of affinity were determined from the negative logarithm of the antagonist dissociation constant (K_B), determined from the equation $K_B = [A]/(DR-1)$ where the dose ratio (DR) was produced by a single concentration of antagonist [A].

In experiments with chloroethylclonidine (CEC), control concentration-response curves to noradrenaline were carried out and the tissues were then exposed to CEC (10 and 100 μM) for 30 min followed by a 60 min washout period, after which a second concentration-response curve to noradrenaline was carried out.

Drugs used in the study

The following drugs were used: [^{3}H]-prazosin from Amersham U.K.; noradrenaline, cocaine hydrochloride, and corticosterone (Sigma, U.K.); chlorethylclonidine, benoxathian, urapidil, 5-methyl-urapidil, WB-4101 (2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane hydrochloride), BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-8-azaspiro [4, 5] decane-7,9-dione dihydrochloride), and spiperone (Research Biochemicals Inc., Semat, U.K.); phentolamine hydrochloride (Ciba-Geigy, Basle, Switzerland); prazosin, doxazosin, alfuzosin, indoramin and SNAP 1069 ([1-(3-benzoylpropyl)-4-benzamidopiperidine dihydrate] were synthesized in the Department of Discovery Chemistry, Pfizer Central Research (Sandwich, U.K.). All other drugs and chemicals were obtained from Sigma (U.K.) or B.D.H. (U.K.). Drugs were dissolved in distilled H_2O or DMSO at 1 mM and subsequent dilutions made in assay buffer.

Results

[^{3}H]-prazosin binding to cloned α_1 adrenoceptor subtypes

The pharmacological profile of cloned rat α_{1D} and human α_{1A}, α_{1B} and α_{1D}-adrenoceptors was assessed by displacement of [^{3}H]-prazosin binding from rat-1 fibroblasts, stably transfected with cDNA encoding each of the α_1 adrenoceptor subtypes. The expression levels of rat and human α_{1D} subtypes were similar (1 pmol mg^{-1}) but were higher for human α_{1A} and α_{1B} subtypes (4–6 pmol mg^{-1}). In each case, analysis of saturation data indicated a single class of high affinity sites. K_d values ($n = 5-6$) were 0.13±0.02 nM for rat α_{1D} and 0.34±0.05, 0.20±0.03 and 0.22±0.05 for human α_{1A}, α_{1B} and α_{1D}-adrenoceptors respectively.

The profile of competing compounds for [^{3}H]-prazosin binding at each α_1-adrenoceptor subtype was determined by displacement of 0.2 nM [^{3}H]-prazosin by at least 12 con-

Table 1 Binding affinities (pK_i) for compounds at cloned human (α_{1A}, α_{1B} and α_{1D}) and rat (α_{1D})-adrenoceptors

	pK_i			
Compound	*Human α_{1A}*	*Human α_{1B}*	*Human α_{1D}*	*Rat α_{1D}*
BMY 7378	6.2±0.10	6.7±0.11	8.2±.010	ND
Prazosin	9.7±0.20	9.6±0.14	9.5±0.10	9.7±0.03
Doxazosin	8.5±0.20	9.0±0.20	8.4±0.12	8.5±0.09
WB 4101	9.3±0.10	8.2±0.16	9.2±0.06	9.4±0.07
5-methyl-urapidil	8.5±0.09	6.8±0.13	7.8±0.09	7.6±0.09
Benoxathian	8.9±0.23	7.8±0.14	8.6±0.12	8.9±0.09
Phentolamine	8.1±0.09	7.1±0.15	7.8±0.03	8.0±0.15
SNAP 1069	7.8±0.19	7.6±0.18	6.8±0.20	ND
Indoramin	8.3±0.03	8.0±0.12	7.3±0.15	7.2±0.10
Alfuzosin	8.0±0.20	8.0±0.13	8.5±0.07	8.5±0.15
Spiperone	7.6±0.12	8.8±0.16	8.1±0.03	8.2±0.15
Urapidil	6.9±0.07	ND	6.9±0.19	6.8±0.03

Affinities were determined by displacement of 0.2 nM [^{3}H]-prazosin from rat-1 fibroblasts stably expressing cloned α_1-adrenoceptor subtypes by 12 concentrations of competing drug. Values represent mean±s.e.mean for 3–5 separate determinations. Hill slopes were not significantly different from unity (ND, not determined).

centrations of competing drug. Displacement affinities (pK_i) are shown in Table 1. Prazosin displayed high and similar affinities (pK_i 9.5–9.7) for all α_1 adrenoceptor subtypes. BMY displayed high affinity for cloned human α_{1D}-adrenoceptors (pK_i 8.2±0.10) and was selective over α_{1A} (pK_i 6.2±0.10) and α_{1B} (pK_i 6.7±0.11) subtypes. Other compounds examined displayed a selective profile for one or more of the receptors studied (Table 1). Consistent with a previous report (Michel *et al.*, 1989) spiperone showed moderate selectivity for α_{1B} over α_{1A} and α_{1D}-adrenoceptors. 5-Methyl-urapidil, WB 4101, benoxathian, and phentolamine were selective for α_{1A} over α_{1B}-adrenoceptors. 5-Methyl-urapidil, indoramin and SNAP 1069 exhibited selectivity for α_{1A} over α_{1D} subtypes.

Displacement affinities for compounds at cloned human and rat α_{1D}-adrenoceptors were very similar (Table 1). Correlation analysis (Figure 1; $r=0.99$, slope = 1.08) confirmed the identical pharmacological profile of compounds for the species homologues of the α_{1D} adrenoceptor.

Effect of competing antagonists

Noradrenaline caused concentration-dependent isometric contractions of rat aorta with a pD_2 of 7.88±0.08 ($n=8$) and a maximum contraction of 1.5–2.0 g. Prazosin, doxazosin and 5-methyl-urapadil shifted noradrenaline concentration-response curves (CRC) to the right and Schild analysis (Figure 2) yielded pA_2 estimates of 9.8±0.30, 8.8±0.08 and 7.8±0.19 respectively. Schild slopes (0.93, 1.02 and 1.04 respectively) were consistent with competitive antagonism. Comparison of the functional potencies for compounds on rat aorta (Table 2) with binding affinity at cloned human α_{1D}-adrenoceptors correlated well (Figure 3a; $r=0.95$). In contrast, functional affinity estimates compared poorly with binding affinities at cloned human α_{1B}-adrenoceptors (Figure 3b; $r=0.61$) and human α_{1A}-adrenoceptors (Figure 3c; $r=0.46$).

The selective α_{1D} antagonist, BMY 7378, also shifted noradrenaline CRC to the right, yielding an apparent pK_B of 8.3±0.1 at 0.03 μM. However, CRC to noradrenaline were not shifted proportionally relative to the increase in BMY 7378 concentration (0.03–1.0 μM). Thus, the slope of the Schild plot was significantly lower than unity (0.62, 95% confidence limits 0.45–0.80, $P<0.05$). Dose-ratios for BMY 7378 (Table 3) were not commensurate with increasing concentrations of antagonist employed and CRC in the presence of BMY 7378 were steeper than corresponding controls.

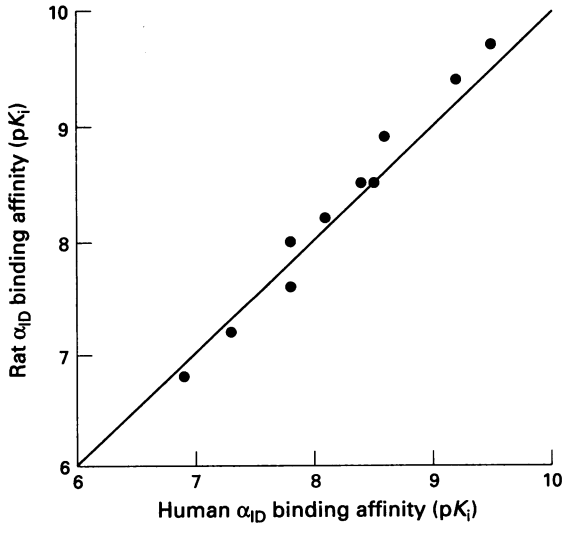

Figure 1 Comparative binding affinities at cloned human and rat α_{1D}-adrenoceptors. Data points are mean binding affinities for the compounds presented in Table 1. Regression analysis ($r=0.99$, slope = 1.1) indicates a very similar pharmacological profile of the two species homologues.

Effect of CEC

Exposure of rat aorta to 10 μM CEC caused a 3 fold dextral shift in the CRC to noradrenaline with no significant reduction in the maximum response. After exposure to 100 μM CEC, noradrenaline curves were shifted further to the right achieving 60% of the maximum response (Figure 4).

Combination experiments

Dose ratios (DR) were determined in the presence of 0.1 μM BMY 7378 and 3 μM indoramin alone or in combination to determine if the two antagonists inhibited noradrenaline-mediated contraction of rat aorta through an interaction at a common site or mutually independent sites (Paton & Rang, 1965). DR analysis (Table 4) indicated that the experimentally

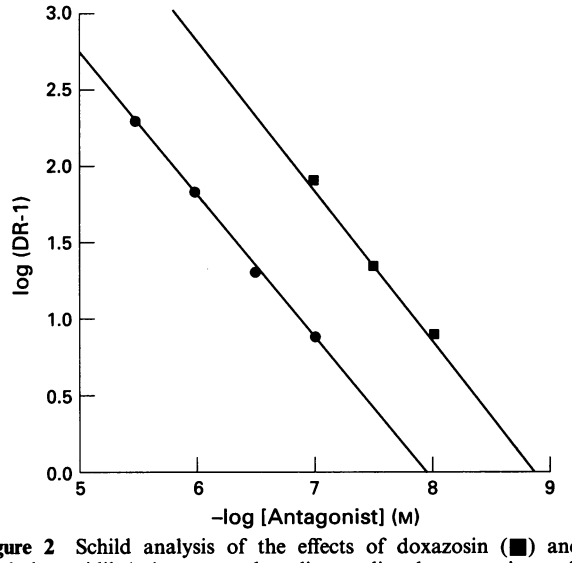

Figure 2 Schild analysis of the effects of doxazosin (■) and 5-methyl-urapidil (●) on noradrenaline-mediated contractions of rat aorta. Each data point represents a separate experiment.

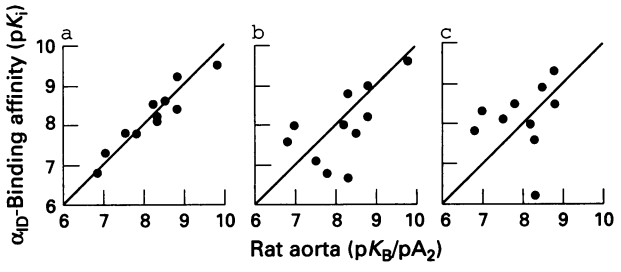

Figure 3 Comparative binding affinities (pK_i values, Table 1) at cloned human α_{1D} (Figure 3a), α_{1B} (Figure 3b), α_{1A} (Figure 3c) adrenoceptors and antagonist affinity estimates on rat aorta (pA_2/pK_B values, Table 3). In each case a theoretical line of equality is shown and regression analysis of the data is indicated in the text.

Table 2 Functional potency of BMY 7378 against noradrenaline-mediated contractions of rat aorta

Concentration of BMY 7378	*Dose-ratio*
0.03 μM	7.3±1.2
0.1 μM	16.7±4.0
0.3 μM	28.7±6.1
1.0 μM	56.9±8.6

Dose-ratios were determined from the shift in the noradrenaline CRC response curve in the presence of BMY 7378 compared to corresponding controls. Values represent the mean±s.e.mean for 4 determinations.

determined combination dose-ratio (38.5 ± 9) was very close to the predicted value from individual dose-ratios for the two antagonists acting at a single site (predicted value 37.5) rather than independent sites (predicted value 337).

Table 3 Functional potencies of antagonists (pA_2 or pK_B) against noradrenaline-mediated contractions of rat aorta

Compound	*pA_2 (slope)*
Prazosin	9.8 ± 0.30 (0.93)
Doxazosin	8.8 ± 0.08 (1.02)
5-methyl-urapidil	7.8 ± 0.19 (1.04)
	pK_B
WB 4101	8.8 ± 0.12
Indoramin	7.0 ± 0.10
Benoxathian	8.5 ± 0.06
Phentolamine	7.5 ± 0.05
Spiperone	8.3 ± 0.06
Alfuzosin	8.2 ± 0.20
SNAP 1069	6.8 ± 0.12

Antagonist potencies (mean ± s.e.mean for 3–4 experiments) were determined as described in methods. pK_B values were determined in the presence of a single concentration of antagonist.

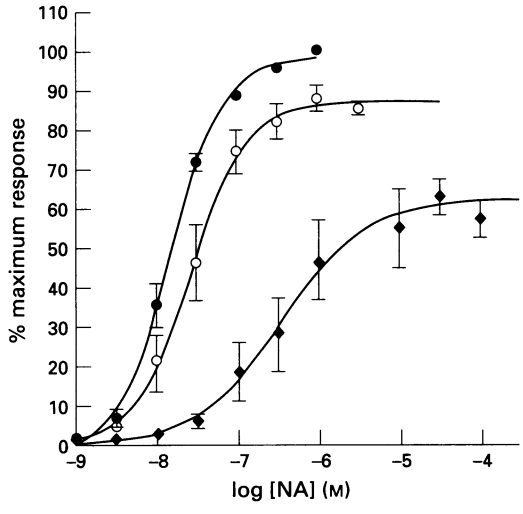

Figure 4 The effect of chlorethylclonidine (CEC) on noradrenaline-mediated contractions of rat aorta. Tissues were exposed to CEC at 10 μM (○) or 100 μM (◆) for 30 min followed by washout for 60 min. Responses are expressed as a percentage of the noradrenaline maximum obtained from the first control curve (●).

Table 4 Dose-ratio analysis of noradrenaline-mediated contractions of rat aorta with BMY 7378 and indoramin

Compound	*Mean dose-ratio*
BMY 7378 (0.1 μM)	12.9 ± 3.3
Indoramin (3 μM)	25.9 ± 6.3
BMY 7378 (0.1 μM) + 5-methyl-urapidil (3 μM)	38.5 ± 9.0
Predicted single site ($DR_1 + DR_2$)-1	37.8
Predicted independent site ($DR_1 \times DR_2$)	334.1

Dose-ratios were determined from 4–5 separate experiments, with all determinations carried out in parallel: means ± s.e.mean are given. Experimentally derived values are given in comparison to predicted values for the compounds acting at a single site or exclusively at independent sites.

Discussion

A number of different studies have attempted to characterize the α_1-adrenoceptor subtype(s) mediating the contractile response of the rat aorta, although several different conclusions have been reached. Some studies suggest that the α_1-adrenoceptor population is a homogeneous entity (Mir & Fozard, 1990; Eltze & Boer, 1992; Aboud *et al.*, 1993) which has been postulated to be either an α_{1B} subtype (Eltze & Boer, 1992) or a receptor which cannot be classified as either α_{1A} or α_{1B} (Mir & Fozard, 1990; Aboud *et al.*, 1993). Other studies have concluded that a number of different α_1 subtypes contribute to the response (Piascik *et al*, 1991; 1994; van der Graaf *et al.*, 1993). On the basis of the data presented in this paper, our results support the contention suggested in some of these studies (Mir & Fozard, 1990; Aboud *et al.*, 1993) that the contractile response to noradrenaline is mediated by a non α_{1A}, non α_{1B} subtype. Indeed, our data suggest that the contractile response to this agonist is predominantly mediated by an α_{1D}-adrenoceptor.

Functional affinity estimates on rat aorta determined with noradrenaline were highly correlated with binding affinities determined at the cloned α_{1D}-adrenoceptor. These data are consistent with the preliminary report of Saussy *et al.* (1994) using phenylephrine as an agonist. This conclusion could be drawn on the basis of binding data in the present study obtained with either cloned rat or human α_{1D}-adrenoceptors. Recently reported studies (Kenny *et al.*, 1994b; Schwinn *et al.*, 1995) show only minor differences between cloned α_1-adrenoceptors from different species. [+]-Niguldipine appears to be an exception having relatively high affinity for human and rat α_{1A}-adrenoceptors compared to the bovine homologue in some (Forray *et al.*, 1994b; Weinberg *et al.*, 1994) but not all (Schwinn *et al.*, 1995) studies. This has enabled reconciliation between the cloned and native α_{1A} subtype (Faure *et al.*, 1994; Ford *et al.*, 1994; Hieble *et al.*, 1995). In contrast to cloned α_{1D}-adrenoceptors, binding data with cloned α_{1A} and α_{1B} subtypes correlated poorly with functional affinity determinations on rat aorta. Indeed, compounds with varying degrees of subtype selectivity inhibited contractions of the aorta with binding affinities only consistent with those determined at the α_{1D} subtype (most notably with BMY 7378, indoramin, 5-methyl-urapidil and SNAP 1069).

It is difficult to compare binding data with cloned α_{1D}-adrenoceptors obtained in our study with the binding characteristics of a homogeneous population of native α_{1D}-adrenoceptors since this has not been described to date. The presence of α_{1D}-adrenoceptors has been suggested in some heterogeneous tissue populations such as rat kidney (Michel *et al.*, 1993). Some studies have suggested that following CEC treatment of rat cortex, the population of sites is characteristic of either α_{1D} (Kenny *et al.*, 1994a) or α_{1A}-adrenoceptors (Eltze & Boer, 1992), although it seems probable that the population of sites following CEC in this tissue is heterogeneous since discriminatary compounds such as (+)-niguldipine reveal multiple sites after CEC treatment (Han & Minneman, 1991; Ford *et al.*, 1994). Nevertheless, the pharmacological profile of cloned rat and human α_{1D}-adrenoceptors obtained in the present study clearly differs from the characteristics of α_{1A} and α_{1B}-adrenoceptors defined in tissues such as rat submaxillary gland and rat liver (Faure *et al.*, 1994).

Our data indicate that the contractile response of the rat aorta in response to noradrenaline is predominantly mediated by a single receptor, namely on α_{1D} subtype, and this is also supported by analysis of combination experiments with BMY 7378 and indoramin. We characterized the effect of BMY 7378 and indoramin in combination, using an indoramin concentration sufficiently high to antagonize any putative α_{1A} or α_{1B} adrenoceptors present. Dose-ratio analysis of the data for the antagonists alone and in combination was consistent with the presence of a single site for which both antagonists mutually competed. This approach was also taken by Eltze & Boer (1992) using WB-4101 and 5-methyl-urapidil,

and they also concluded that a single subtype mediated the response to noradrenaline. However, this finding may not hold at higher antagonist concentrations, typically evoking much larger agonist dose-ratios and may be limited by the relative contribution of different receptor subtypes. Alternatively, another site may be present which is not effectively antagonized by either BMY 7378 or indoramin.

It could be argued that the CEC sensitivity of the contractile response of rat aorta might be indicative of α_{1B}-adrenoceptors (Eltze & Boer, 1992); however, this conclusion is not supported by the pharmacological profile of any of the compounds examined. Both rat and human homologues of the cloned α_{1D}-adrenoceptor show a moderate degree of sensitivity to alkylation by CEC, greater than the α_{1A} subtype, but to a lesser extent than the α_{1B} subtype (Forray *et al.*, 1994a,b; Schwinn *et al.*, 1994). The effect of CEC on the rat aorta is consistent with the sensitivity of the cloned α_{1D}-adrenoceptor to CEC and contrasts with the reported insensitivity of the α_{1A} subtype in smooth muscle preparations (Bylund *et al.*, 1994). However, it is often difficult to reconcile findings with CEC as a means of differentiating between subtypes since alkylation by CEC is dependent on species, time of exposure and nature of the preparation which makes comparison of different experimental protocols difficult. Thus, the component of the noradrenaline response that was resistant to CEC in our studies could indicate incomplete alkylation of the α_{1D}-adrenoceptor or the presence of a CEC resistant subtype(s). Analysis of antagonist profiles following CEC treatment would provide some insight into the nature of any additional component. It is interesting to note that some α_1-adrenoceptor subtypes which cannot be reconciled with currently identified cloned adrenoceptors are relatively insensitive to CEC (α_{1L}-adrenoceptors; Muramatsu *et al.*, 1990).

The exclusive presence of mRNA for the α_{1D}-adrenoceptor has been suggested in rat aorta on the basis of Northern analysis (Lomasney *et al.*, 1991) although more recent *in situ* hybridization studies have indicated the presence of all three α_1 subtypes (Piascik *et al.*, 1994). Heterogeneity of α_1-adrenoceptors has also been claimed on the basis of [^{3}H]-prazosin binding to rat aorta membranes in which compounds such as WB 4101 and 5-methyl-urapidil exhibit two different binding affinities (Piasik *et al.*, 1994). Piascik *et al.* (1991) also reported that the concentration-response curve to phenylephrine following CEC treatment became biphasic, indicating the presence of two functional α_1-adrenoceptors, only one of which was CEC-sensitive. Further evidence for heterogeneity of α_1-adrenoceptors on rat aorta has also been claimed on the basis of other functional studies (van der Graaf *et al.*, 1994) in which noradrenaline concentration-response curves in the presence of several antagonists could not be accounted for by simple competitive antagonism at a single receptor (Schild slopes lower than unity and steepening of agonist concentration-response curve). Cellular signalling pathways have previously been used as an additional criterion for discriminating between subtypes and in rat aorta both dependence and independence of the α_1-mediated contractile response on extracellular Ca^{2+} has been demonstrated (Mir & Fozard, 1990; Oriowo *et al.*, 1990). However, differential coupling to signalling mechanisms cannot be reliably used to classify α_1-adrenoceptor subtypes (Minneman, 1988) and in rat aorta since antagonist potencies were unaffected by the presence of nifedipine, both intracellular Ca^{2+} increases and dihydropyridine-sensitive calcium entry can be attributed to activation of a single subtype (Mir & Fozard, 1990; Ruffolo *et al.*, 1991).

Thus, whilst in our studies evidence indicates that a single subtype predominantly mediates the contractile response to noradrenaline, we cannot preclude the existence of other subtypes. Using the selective α_{1D} antagonist BMY 7378, pA_2 determinations consistently yielded Schild slopes less than unity (slope = 0.62). Dose-ratios were not commensurate with increasing concentrations of BMY 7378 and noradrenaline curves were steepened in the presence of the antagonist. This finding is consistent with the contention that noradrenaline activates more than one receptor, although this profile was not exhibited by other antagonists in our and other studies (Eltze & Boer, 1992; Aboud *et al.*, 1993), nor is it supported by data derived from combination studies.

A number of studies with cloned human and other mammalian α_1-adrenoceptors indicate that noradrenaline has higher affinity for the cloned α_{1D} subtype in comparison to other subtypes (Perez *et al.*, 1991; Michel & Insel, 1994). Since noradrenaline displays high affinity on rat aorta (possibly reflecting a large receptor reserve), it may be possible that this agonist is relatively selective for α_{1D}-adrenoceptors under the conditions used in our study, to the extent that activation of other subtypes occurs only at much higher agonist concentrations. This could account for steepening of noradrenaline curves and low Schild slope with BMY 7378. Taken together, although our data suggest a predominant role for α_{1D}-adrenoceptors in the contractile response to noradrenaline, consistent with other reports suggesting that a single subtype mediates the contractile response, we cannot fully exclude the presence of other subtypes.

In summary, we have shown that the pharmacological profile of compounds at cloned human and rat α_{1D}-adrenoceptors is consistent with functional affinity estimates determined on rat aorta. The selective α_{1D} antagonist, BMY 7378, also antagonized noradrenaline-mediated contractions of rat aorta consistent with α_{1D} binding affinity although the profile of this compound did not suggest competitive antagonism at a single receptor. The data in our study suggests that noradrenaline predominantly contracts the rat aorta through an α_{1D}-adrenoceptor, but the involvement of other subtypes cannot be precluded at the present time.

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