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Pharmacological characterization of an α_{1A}-adrenoceptor mediating contractile responses to noradrenaline in isolated caudal artery of rat

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1 The α_1-adrenoceptor population mediating contraction of caudal artery of rat has been characterized by using quantitative receptor pharmacology.

2 Cumulative concentration-effect (E/[A]) curves to noradrenaline (NA) yielded a $p[A]_{50}$ of 5.56 ± 0.05 ($n = 16$). Prazosin caused concentration-dependent, parallel, dextral shifts of E/[A] curves to NA yielding a pK_b of 8.9 (Schild regression slope = 1.0). RS-17053 (N-[2-(2-cyclopropyl methoxy phenoxy) ethyl]-5-chloro-α ,α-dimethyl -1H-indole- 3-ethanamine hydrochloride; 10 – 100 nM), a selective α_{1A}-adrenoceptor antagonist, produced non-parallel, biphasic, dextral shifts of E/[A] curves to NA, suggesting the involvement of more than one α_1-adrenoceptor subtype. Analysis of the high affinity component yielded an apparent pA_2 value of 9.2 ± 0.3.

3 A-61603, a selective agonist at α_{1A} adrenoceptors behaved as a full agonist relative to NA and yielded monophasic E/[A] curves with a $p[A_{50}]$ of 7.59 ± 0.04 ($n = 15$). Pretreatment of tissues with chloroethylclonidine (CEC; 100 μM for 20 min, followed by 40 min washout), which preferentially alkylates α_{1B}- and α_{1D}-adrenoceptors, did not alter E/[A] curves to A-61603. Prazosin (3 – 300 nM) caused concentration-dependent, parallel, dextral shifts of E/[A] curves to A-61603 yielding a pA_2 estimate of 9.2 ± 0.2.

4 Experiments with α_1-adrenoceptor antagonists of varying subtype selectivities (RS-17053, SNAP 5089, tamsulosin, 5-methylurapidil, BMY 7378, HV 723 and REC 15/2739) revealed parallel dextral shifts of E/[A] curves to A-61603. Schild regression analyses yielded pA_2 estimates of 9.2, 9.3, 11.2, 9.0, 6.3, 8.7 and 10.0 for RS-17053, SNAP 5089, tamsulosin, 5-methylurapidil, BMY 7378, HV 723 and REC 15/2739, respectively, although deviations from unit slope (possibly reflecting a secondary involvement of another α_1-adrenoceptor) hindered estimations of pK_b for some antagonists. The antagonist affinity profile obtained reflects best that described for the α_{1A}-adrenoceptor.

5 In conclusion, caudal artery of rat contracts in response to NA via activation of at least two α_1-adrenoceptor subtypes. One of these subtypes displays the pharmacology of the α_{1A}-adrenoceptor, while the other remains to be defined. Use of the novel selective agonist, A-61603, allows for limited pharmacological isolation of the α_{1A}-adrenoceptor permitting characterization of the properties of selective antagonists.

Keywords: Blood vessels; RS-17053; A-61603; SNAP 5089; prazosin, α_1-adrenoceptor

Introduction

For over a decade, pharmacological studies have demonstrated heterogeneity among α_1-adrenoceptors, prompting regular revisions to classification schemes (see Ford *et al*., 1994; Bylund *et al*., 1994). Currently, classification of α_1-adrenoceptors recognises three subtypes (α_{1A}, α_{1B}, and α_{1D}; Hieble *et al*., 1995a). In addition, the existence of a fourth α_1-adrenoceptor, the putative α_{1L}-adrenoceptor (Muramatsu *et al* 1990a,b; Oshita *et al*., 1993), has been proposed on the basis of functional studies, although a molecular biological correlate (e.g., distinct gene) for this adrenoceptor is lacking.

Particular interest has focused recently on the identity of the α_1-adrenoceptor which functions to contract smooth muscle of the human lower urinary tract (prostate, bladder neck and urethra; see review, Hieble *et al*., 1995b). Several groups claim that the α_{1A}-adrenoceptor is involved, especially as its mRNA predominates in these tissues (Faure *et al*., 1994; Forray *et al*., 1994; Marshall *et al*., 1995). Others contend that the α_{1L}-adrenoceptor is involved. This contention is based on low affinity estimates for several antagonists, including prazosin, SNAP 5089 and RS 17053 (Muramatsu *et al*., 1994; Ford *et al*., 1996a). These probes serve to distinguish the α_{1L}-adrenoceptor from the α_{1A}-adrenoceptor. However, the validity of this distinction for tissues of the human lower urinary tract rests on experiments done solely in isolated tissue baths ('static' tissue preparations). Consequently, it has been argued that the usefulness of these defining pharmacological probes (prazosin, SNAP 5089, RS 17053, etc.) should be confirmed by an α_{1A}-adrenoceptor bioassay performed under identical methodological conditions (cumulative concentration-effect (E/[A]) curves in a tissue bath).

A functional bioassay for the α_{1A}-adrenoceptor was first described in the rat vas deferens (Han *et al*., 1987; Aboud *et al*., 1993; Burt *et al*., 1995). However, despite being performed in static tissue baths, this assay is not ideal as contractile responses to noradrenaline (NA) are complex and phasic, and analyses are limited to construction of non-cumulative E/[A] curves. High affinity competitive antagonists such as tamsulosin cause suppression of curve maxima (apparent insurmountability) under these conditions (Furukawa *et al*., 1995b), precluding affinity determinations. Furthermore, evidence exists to question whether a singular, homogeneous α_{1A}-adrenoceptor population functions in this tissue (Ohmura *et al*., 1992). Functional α_{1A}-adrenoceptor populations have been defined more thoroughly in perfused vascular beds from rat, including isolated kidney (Eltze & Boer 1992; Blue *et al*., 1995) and isolated mesentery (Williams & Clarke, 1995). However, these assays are methodologically quite dissimilar to those

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performed on tissues from human lower urinary tract making direct comparisons difficult. In addition, as these functional assays only allow construction of non-cumulative E/[A] curves, their suitability for analysis of high affinity antagonists (e.g., tamsulosin, S-niguldipine) is limited (Blue *et al*., 1995). Indeed, consideration of the literature reveals that an unequivocal α_{1A}-adrenoceptor, in a static tissue bath preparation, has not been described. In this regard, we now describe studies using helical strips of caudal artery of rat in isolated tissue baths and cumulative E/[A] curves. Although it has been shown previously that noradrenaline (NA) mediates contraction in this blood vessel by activation of more than one α_1-adrenoceptor subtype (Medgett & Langer, 1984) as well as putative activation of postjunctional α_2-adrenoceptors (Templeton *et al*., 1989; MacLean & McGrath, 1990), a comprehensive characterization with a range of subtype-selective pharmacological probes has not been described.

The results indicate that caudal artery of rat contracts in response to NA via activation of at least two α_1-adrenoceptor subtypes, one of which displays the pharmacology of the α_{1A}-adrenoceptor. Use of the novel selective α_{1A}-adrenoceptor agonist, A-61603, allowed for partial pharmacological isolation (30 fold window) of this receptor for analytical studies. Data obtained with A-61603 confirm the usefulness of antagonists such as RS-17053 and SNAP 5089 for the pharmacological characterization of α_1-adrenoceptors (most notably distinguishing α_{1L}- and α_{1A}-adrenoceptors) in 'static' tissue bath preparations. A preliminary account of some of these studies has been presented previously (Lachnit *et al*., 1995).

Methods

Tissue preparation

Male Sprague Dawley rats (200–350 g) were killed by asphyxiation with CO_2. The caudal artery was removed carefully and cleaned of adhering tissue. The endothelium was removed and the artery cut into helical strips (5–10 mm long, 1–2 mm wide). Strips were suspended in waterjacketed tissue baths (37°C) for measurement of isometric tension in oxygenated (95% O_2, 5% CO_2) Krebs solution containing (mM): Na^+ 143.5, K^+ 6.0, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 125.8, HCO_3^- 25, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, supplemented with 100 μM ascorbate (to minimize oxidation of NA), 30 μM cocaine, 30 μM corticosterone (to block neuronal and extraneuronal uptake of NA, respectively), 10 μM indomethacin (to inhibit prostanoid production), 1 μM propranolol (to block β-adrenoceptors), and 300 nM idazoxan (to block α_2-adrenoceptors). Resting tension was set at approximately 4 mN at the beginning of the experiment and tissues were equilibrated for 30 min.

Agonist studies

Following the construction of a concentration-effect curve to NA (cumulative addition in 0.5 log M increments) and a 45 min washout period, concentration-effect curves to agonists were obtained by cumulative addition (0.5 log increments) of agonist.

Antagonist studies

With the exception of chloroethylclonidine (CEC), all experiments with antagonists were performed as follows. Following construction of a control agonist concentration-effect curve, each tissue was incubated for 60 min with antagonist. A second curve to the agonist was then constructed.

Inactivation studies

Experiments with CEC were performed as follows. After construction of a control concentration-effect curve to NA or A-61603, each tissue was incubated with CEC (100 μM) for 20 min. Tissues were then washed regularly for 40 min before construction of a second concentration-effect curve to either NA or A-61603.

Data analysis

Concentration-effect curves were plotted by use of non-linear iterative curve-fitting methodologies to a form of the logistic equation for estimation of mid-point location parameter ($[A]_{50}$), such that $E = E_{max} \bullet [A]^{nH}/([A]^{nH} + [A]_{50}{}^{nH})$, where E_{max} is the magnitude of the upper asymptote and n_H is the Hill coefficient (defining the slope of the relationship). Antagonist affinity estimates (as pK_b or pA_2) were obtained by construction of Schild regressions, except in the case of RS-17053 versus NA, which was obtained by 'single-concentration' analysis (assuming a Schild regression slope of 1) according to the equation: $pA_2 = -\log[B] + \log(r-1)$, where [B] is molar concentration of antagonist, and r is the ratio of $[A]_{50}$ in the presence of RS-17053 divided by that obtained in the absence of RS-17053. Wherever possible, estimates of r used in Schild regressions were corrected for variations in tissue sensitivity to agonist over time. Data presented are means ± s.e.mean and *n* represents number of tissues. All terms and equations used in this study are in accordance with IUPHAR guidelines (Jenkinson *et al*., 1995).

Materials

Methoxamine hydrochloride, chloroethylclonidine hydrochloride, 5-methylurapidil, cocaine hydrochloride, idazoxan hydrochloride, prazosin hydrochloride, and BMY 7378 dihydrochloride (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9-dione dihydrochloride) were obtained from Research Biochemicals Inc. (Natick, MA, U.S.A.). (±)-Noradrenaline hydrochloride, (±)-propranolol hydrochloride, phenylephrine, and corticosterone were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Fluparoxan hydrochloride was obtained from Glaxo. Amidephrine, (±)-SDZ NVI 085 (3,4,4a5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2H-naphth [2,3-b]-1,4-oxazine hydrochloride), HV-723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxy phenoxyethyl)-amino)-propyl)benzene-aceto-nitrile) fumarate), A-61603 (±N-[5-(4,5-dihydro-1 H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methanesulphonamide hydrobromide), RS-17053 (N-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α,α,-dimethyl-1H-indole-3-ethanamine hydrochloride), REC 15/2739 (SB 216469; 8-3-[4-(2-methoxyphenyl)-1-piperazinyl]-propylcarbamoyl)-3-methyl-4-oxo-2-p-henyl-4H-1-benzopyran dihydrochloride), SNAP 5089 (2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid (N[3-(4,4-diphenylpiperidin-1-yl)propyl]amide methyl ester), NS-49 ([**R**]-(−)-3′-(2-amino-1-hydroxyethyl)-4″-fluoromethane sulphonanilide hydrochloride) and tamsulosin (YM 617) were synthesized in the Chemistry department at Roche Bioscience (Palo Alto, CA, U.S.A.). Solutions were prepared in deionized water or dimethylsulphoxide (corticosterone, prazosin, RS-17053, tamsulosin, HV-723, REC 15/2739, SNAP 5089, 5-methyl urapidil, and (±)-SDZ NVI 085). The final concentration of dimethylsulphoxide in the bathing solution did not exceed 0.1% and had no effect on muscle contraction.

Results

Increasing concentrations (0.5 log increments) of NA produced sustained concentration-dependent increases in tension in caudal artery of rat. E/[A] curves were monophasic ($n_H = 1.0 \pm 0.07$) and yielded a $p[A]_{50}$ estimate for NA of 5.56 ± 0.05 (mean ± s.e.mean; $n = 16$). Prazosin (3–300 nM) produced parallel dextral shifts of E/[A] curves to NA without a change in maxima (Figure 1a). Schild regression analysis yielded a slope of 1.0 and a pK_B estimate of 8.9 (Figure 1b). In

contrast to prazosin, RS 17053 (10, 30 and 100 nM), a novel, selective α_{1A}-adrenoceptor antagonist (Ford *et al*., 1996a), produced surmountable non-parallel, dextral shifts of E/[A] curves to NA, which could be resolved into two sites by use of biphasic analysis (assuming n_H of 1.0 for each phase; Figure 2a). Schild regression analysis of the 'high-affinity' site yielded an apparent pA_2 value for RS-17053 of 9.2 ± 0.3 ($n = 15$). Exposure of tissues to 100 μM CEC for 20 min (which preferentially alkylates α_{1B}- and α_{1D}-adrenoceptors; Han *et al*., 1987; Hieble *et al*., 1995a) did not significantly influence E/[A] curves to NA (Figure 2b). However, Figure 2b also shows that following CEC treatment, RS-17053 (30 nM) elicited surmountable parallel dextral shifts of E/[A] curves to NA (pA_2 estimate of 8.6; $n = 4$). In tissues pretreated with RS 17053 (100 nM) to block α_{1A}-adrenoceptors, BMY 7378 (10–100 nM), a selective α_{1D}-adrenoceptor antagonist (Saussy *et al*., 1994), failed to shift E/[A] curves to NA, implicating no role for α_{1D}-adrenoceptors.

The α_{1A}-adrenoceptor selective agonist, A-61603, elicited robust increases in tension that did not fade over time (Figure 3) and behaved as a full agonist relative to NA. As can be seen from Figure 3, the absence of response 'fade' in this assay system was well reflected by the ability of A-61603 to surmount fully the antagonism produced by tamsulosin, a slowly dissociating antagonist. More time was needed to reach steady-state in the A-61603 E/[A] curve in tissues equilibrated with tamsulosin (Figure 3d) than was needed to achieve steady-state in the presence of prazosin (Figure 3b) and RS-17053 (Figure 3c). Figure 4 shows that cumulative additions (0.5 log increments) of A-61603 resulted in monophasic E/[A] curves ($n_H = 1.05 \pm 0.06$) with a $p[A]_{50}$ of 7.59 ± 0.04 ($n = 15$) and that, unlike the effect on responses to NA (Figure 2), RS-17053 (3–300 nM) evoked surmountable parallel dextral shifts of E/[A] curves to A-61603. Further studies also revealed that E/[A] curves to A-61603 constructed in the presence of fluparoxan (1 μM), an α_2-adrenoceptor antagonist with more than 2500 fold selectivity over α_1-adrenoceptors (Halliday *et al*., 1991) were not significantly different from those constructed in the presence of 300 nM idazoxan (data not shown), providing evidence that α_2-adrenoceptors were not functionally involved in responses to A-61603. Additionally, incubation with CEC (100 μM for 20 min followed by washout) was without effect on E/[A] curves to A-61603.

Prazosin (3–300 nM, produced parallel dextral shifts of E/[A] curves to A-61603 with no significant reduction in maxima. In the presence of 1 μM prazosin, the α_2-adrenoceptor antagonist fluparoxan (3–30 μM) failed to shift E/[A] curves to A-61603 any further (data not shown), illustrating that even after E/[A] curve displacement, α_2-adrenoceptor involvement was not evident.

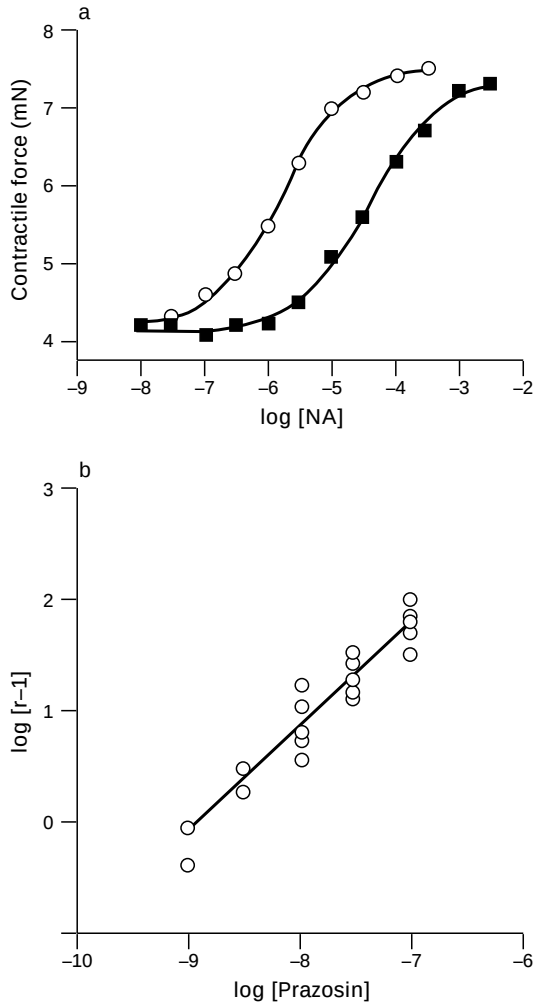

Figure 1 (a) The effect of prazosin on contractile responses to NA in isolated caudal artery of rat. A representative concentration-effect curve which was repeated five times is shown for NA in the absence (○) and in the presence (■) of 30 nM prazosin. (b) Schild regression for antagonism of NA-induced contraction of caudal artery by prazosin. $pA_2 = 8.9$ (slope = 1.0; 95% confidence limits = 0.85–1.14). Each point represents a single determination.

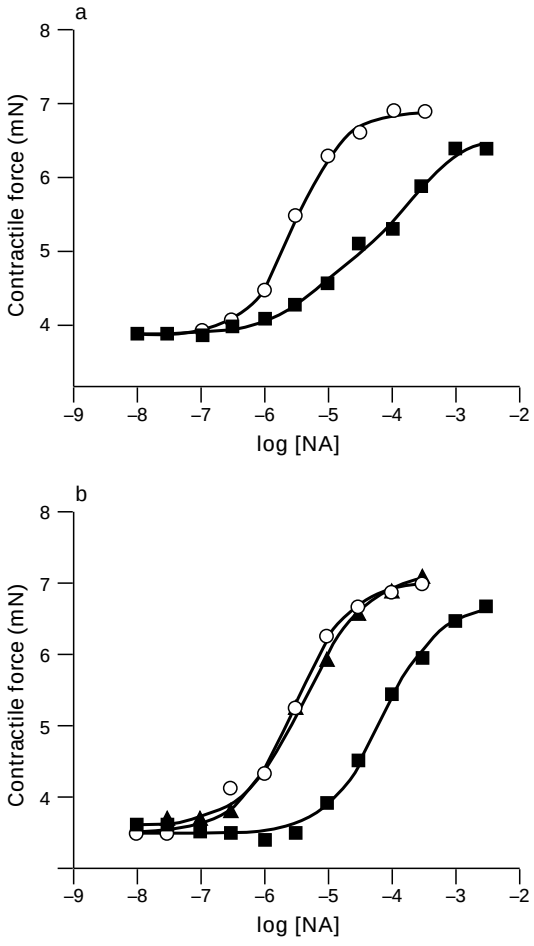

Figure 2 (a) The effect of RS-17053 on contractile responses to NA in isolated caudal artery of rat. A representative concentration-effect curve which was repeated six times is shown for NA in the absence (○) and in the presence (■) of 30 nM RS-17053. (b) The effect of chlorethylclonidine (CEC) treatment and RS-17053 following CEC treatment on contractile responses to NA in isolated caudal artery of rat. A representative concentration-effect curve which was repeated four times is shown for NA before (○), after (▲) 100 μM CEC treatment (20 min exposure, 40 min washout), and in the presence of 30 nM RS-17053 following CEC treatment (■).

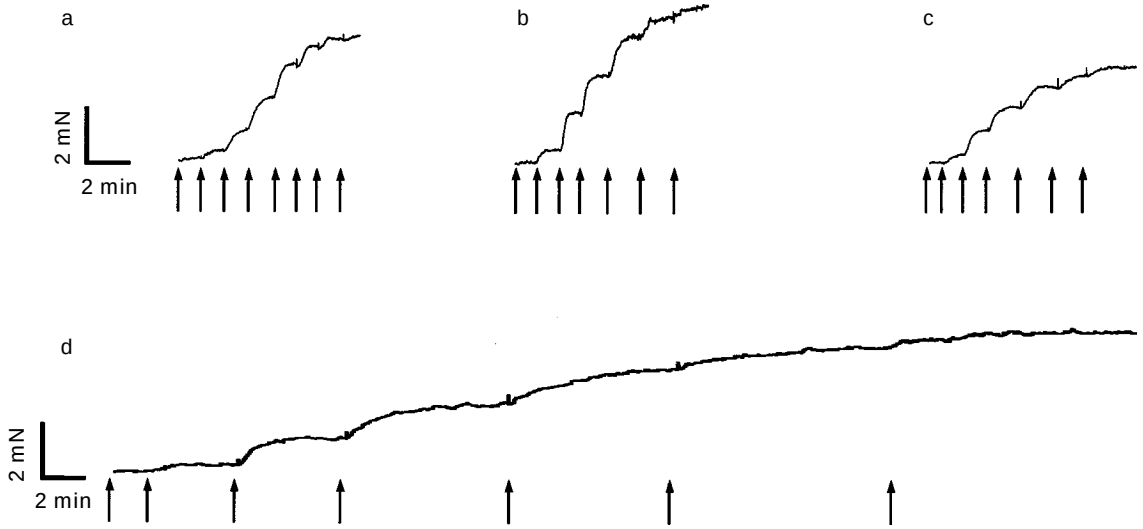

Figure 3 Representative experimental traces showing contractile responses to increasing concentrations of A-61603 in isolated caudal artery of rat under different experimental conditions. Arrows indicate half-log unit concentration increments. (a) Responses to A-616103 in the untreated tissue. (b) Responses to A-61603 in the presence of 30 nM prazosin. (c) Responses to A-61603 in the presence of 30 nM RS-17053. (d) Responses to A-61603 in the presence of 3 nM tamsulosin. Time intervals (shown) are the same for each trace.

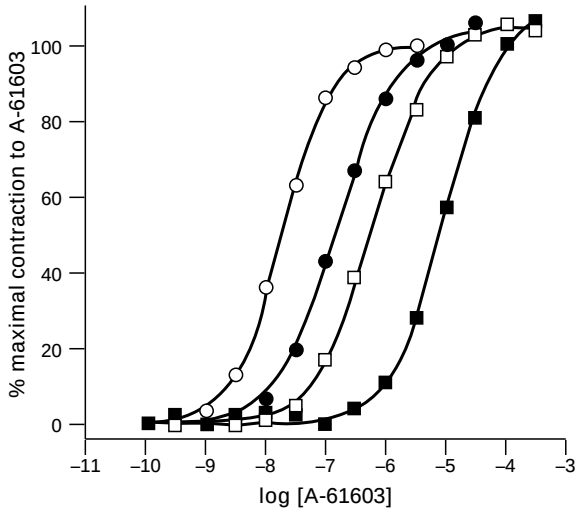

Figure 4 Concentration-effect curve to A-61603 in isolated caudal artery of rat. A representative concentration-effect curve is shown for A-61603 which was repeated five to six times in the absence (○) and presence of 3 nM (●), 30 nM (□), and 300 nM (■) RS-17053. Values presented are expressed as a percentage of the maximal contraction of the curve in the absence of RS-17053.

Similarly, the high affinity α_{1A}-adrenoceptor antagonists, RS-17053 (3–300 nM), SNAP 5089 (10–300 nM), 5-methylurapidil (10–300 nM), tamsulosin (10–300 nM), and REC 15/2739 (10–300 nM) all produced parallel dextral shifts of E/[A] curves to A-61603 with no significant change in maxima. As is evident in Table 1 and Figure 5, Schild regression slopes for tamsulosin, HV 723, SNAP 5089, BMY 7378, and oxymetazoline were not significantly different from 1, consistent with simple competitive antagonism at a single receptor population. In contrast, Schild regression analysis for prazosin, RS 17053, 5-methylurapidil, and REC 15/2739 yielded lines with slopes that were slightly, but significantly, less than 1 (Table 1 and Figure 5. However, in each case, it was possible to analyse a component of the Schild regression which was consistent with a competitive interaction at a single population of α_1-adrenoceptors (bold line in Figure 5 with concentration-ratios of up to approximately 20 to 30 fold). The estimates of antagonist affinity (pK_b) and slopes for these partial regressions are also given in Table 1.

An agonist potency profile was obtained in order to extend the characterization of the α_1-adrenoceptor involved in contraction of rat caudal artery. Table 2 shows that, with the exception of A-61603 and phenylephrine, all agonists acted as partial agonists relative to NA. The following potency order (relative potency) was observed: A-61603 (0.01) > > NA (1) = phenylephrine (1) > SDZ NVI 085 (2.3) = NS-49 (2.4) > amidephrine (6) = methoxamine (6). E/[A] curves for each agonist were displaced in a parallel manner, and with high affinity, by RS-17053 (30 nM).

Oxymetazoline, although eliciting increases in tension, did not act as an α_1-adrenoceptor agonist, as RS-17053 (3–300 nM) and prazosin (3–300 nM) failed to antagonize responses to oxymetazoline (data not shown). Contractile responses to oxymetazoline were not antagonized by fluparoxan (1 μM), but were antagonized by mesulergine (1 μM), and ketanserin (100 nM; data not shown), both of which behave as 5-HT_2 receptor antagonists (Van Wijngaarden *et al*., 1990). As agonism of α_1-adrenoceptors was not apparent with oxymetazoline, its ability to antagonize responses to A-61603 was evaluated. Oxymetazoline (100–300 nM) produced parallel concentration-dependent shifts in the E/[A] curves to A-61603 without affecting maxima, yielding a pK_b estimate of 7.6 (Table 1).

Discussion

Isolated helical strips from caudal artery of rat developed tension in a robust and sustained manner in response to increasing concentrations of NA. Antagonism of NA-mediated responses by prazosin was surmountable and fully consistent with simple competition, as indicated by a Schild regression slope of 1. The subnanomolar affinity estimate for prazosin confirms the involvement of α_1-adrenoceptors in mediating the contraction to NA.

In contrast to prazosin, RS-17053, which is a selective α_{1A}-adrenoceptor antagonist, displaced the E/[A] curves to NA in a biphasic manner, indicating contributions from more than one adrenoceptor. However, after treatment with CEC, RS-17053 evoked only parallel, high affinity shifts. Thus, it appears that CEC inactivated a low affinity site for RS-17053. However, the

role of this putative site in the contractile response to NA, under normal conditions, may be minimal, as the treatment with CEC itself failed to alter E/[A] curves to NA.

Incubation of tissues with BMY 7378 (an antagonist selective for the α_{1D}-adrenoceptor subtype; Goetz *et al*., 1995) at concentrations up to 100 nM, alone and in the presence of RS-17053, did not influence E/[A] curves to NA (data not shown). This indicates that the α_{1D}-adrenoceptor subtype does not contribute substantially to NA-mediated contractions of the rat caudal artery, despite the presence of messenger RNA for this receptor subtype (Piascik *et al*., 1995). Thus, RS-17053 resistant contractions may be mediated by the α_{1B}-adrenoceptor subtype. Further studies with a high affinity competitive antagonist possessing selectivity for the α_{1B}-adrenoceptor will be needed to test this notion.

In order to achieve selectivity of agonism, A-61603, a novel imidazoline molecule was employed. A-61603 is selective for α_{1A}-adrenoceptors and, despite its imidazoline structure, shows only weak agonist activity at α_2-adrenoceptors, (Knepper *et al*., 1995). In caudal artery strips, A-61603 elicited stable increases in tension that did not fade and behaved as a full agonist relative to NA. These sustained increases in tension allowed for cumulative E/[A] curves to be constructed, with a potency value displayed for A-61603 that was only slightly lower than has been found previously (7.70, canine prostate; 8.21, rat vas deferens; Knepper *et al*., 1995). This potency value, when compared with affinity estimates at cloned α_1-adrenoceptor subtypes, is most consistent with activation of α_{1A}-adrenoceptors (Knepper *et al*., 1995). Cumulative E/[A] curves to A-61603 surmounted fully the antagonism of all agents tested, including those antagonists (e.g. tamsulosin, dihydropyridines) which have been shown previously to be insurmountable in other α_{1A}-adrenoceptor assays (see Furukawa *et al*., 1995a,b). Thus, because of the stability of responses in caudal artery, it was possible to make affinity determinations for several anatagonists which might otherwise have appeared 'pseudoirreversible' (see Kenakin, 1993).

Table 1 Affinity estimates for α_1-adrenoceptor antagonists versus A-61603 in isolated caudal artery of rat

	Total regression[a]			*Partial regression*[b]		
Antagonist	pA_2	*Slope (CI)*	n[c]	pK_b[d]	*Slope*	n[c]
Prazosin	9.2	0.83 (0.69–0.98)[e]	14	9.1	1.1	8
RS-17053	9.2	0.84 (0.71–0.98)[e]	24	9.2	1.1	11
5-Methyl-urapidil	9.0	0.83 (0.76–0.90)[e]	13	8.9	0.97	7
Tamsulosin	11.2	0.82 (0.51–1.14)	12	10.9	0.97	9
SNAP 5089	9.5	0.95 (0.64–1.25)	13	9.4	1.1	8
REC 15/2739	10.0	0.78 (0.64–0.91)[e]	18	9.8	1.2	8
HV 723	8.7	1.1 (0.89–1.28)	15	8.8		
BMY 7378	6.3	0.99 (0.54–1.43)	6	6.3		
Oxymetazoline	7.8	0.84 (0.41–1.26)	12	7.6		

[a]Schild analysis conducted on all data points. [b]Schild analysis conducted on the first 'linear' portion of the regression. See Figure 5. [c]Number of observations. [d]Estimated with slope constrained to 1. [e]Significantly different from 1 ($P<0.05$).

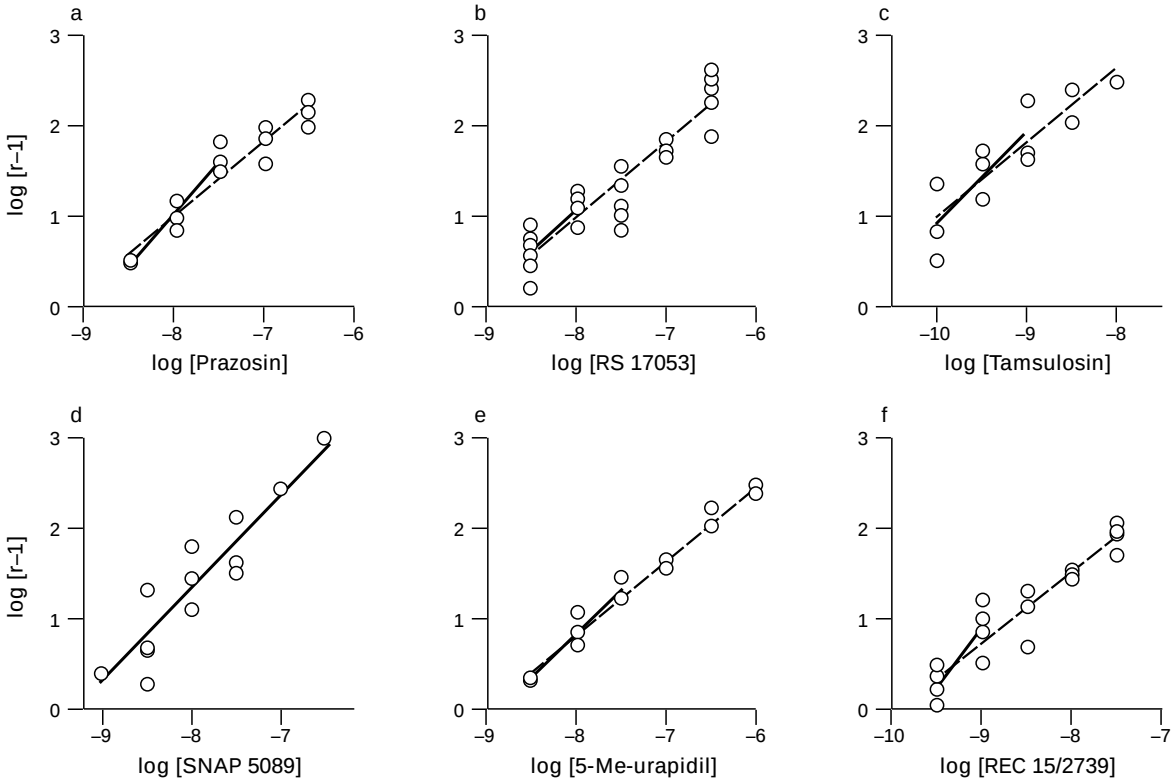

Figure 5 Schild regression for antagonism of A-61603-induced contraction of the isolated caudal artery of rat by (a) prazosin, (b) RS-17053, (c) tamsulosin, (d) SNAP 5089, (e) 5-methylurapidil and (f) REC 15/2739. Each point represents a single determination. Stippled line represents Schild regression through all data points. Bold line represents Schild regression conducted on the first linear portion of the regression. For pA_2 values and Schild slopes see Table 1.

Table 2 Relative potency and intrinsic activity of α_1-adrenoceptor agonists in isolated caudal artery of rat

Agonist	*Max. response*[a]	*p[A50]*[b]	n[c]
A-61603	100%	7.59±0.04	15
Noradrenaline	100%	5.56±0.05	16
Phenylephrine	100%	5.42±0.20	8
SDZ NVI 085	36%	5.27±0.18	4
NS 49	59%	5.05±0.18	4
Amidephrine	49%	4.76±0.07	4
Methoxamine	63%	4.73±0.12	4
Oxymetazoline[d]	ND[e]	6.66±0.10	15

[a]Relative to maximum response to NA. [b]Mean±s.e.mean. [c]Number of determinations. [d]Not mediated via α_1-adrenoceptors based on insensitivity of responses to prazosin and RS-17053. [e]Not determined.

Table 3 Summary of affinity estimates for α_1-adrenoceptor ligands in isolated caudal artery of rat and cloned α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors taken from the literature

	Rat caudal artery[a] pK_b	α_{1A} pK_i	α_{1B} pK_i	α_{1D} pK_i
Prazosin	9.1	9.9	10.1	9.9
RS-17053	9.2	9.5	7.8	7.8
5-Methyl-urapidil	8.9	9.4	7.5	8.0
Tamsulosin	10.9	10.4	9.3	10.2
SNAP 5089	9.4	9.5	6.9	6.8
REC 15/2739[b]	9.8	9.5	7.3	7.6
HV 723	8.8	9.6	9.7	8.5
BMY 7378[c]	6.3	6.1	6.2	8.2
Oxymetazoline[d]	7.6	7.8	6.4	6.5
'All'[e]		$r^2=$ 0.88	$r^2=$ 0.06	$r^2=$ 0.50

[a]pK_b values versus A-61603 in isolated caudal artery of rat taken from Table 1. Literature values shown from Ford *et al*., 1996a,b; except: [b]Testa *et al*., 1995; [c]Goetz *et al*., 1995; [d]Michel *et al*., 1993. [e]Coefficient of correlation: caudal artery versus cloned receptor.

Table 3 shows that compounds with selectivity for the α_{1A}-adrenoceptor, most notably RS-17053, SNAP 5089, REC 15/2739, and 5-methylurapidil, inhibited A-61603-mediated contractions of rat caudal artery with high affinity (pK_b estimates of 8.9–9.8), consistent with an action at the α_{1A}-adrenoceptor subtype (Table 3). Moreover, BMY 7378 exhibited low affinity consistent with that observed previously at α_{1A}-adrenoceptors (Saussy *et al*., 1994). The high affinities obtained with RS-17053 and SNAP 5089 indicate clearly that these antagonists can disclose α_{1A}-adrenoceptors under conditions which prevail in isolated tissue bath experiments.

Agonist potencies in rat caudal artery are similar to those obtained previously in the perfused isolated kidney of rat (Eltze & Boer, 1992; Blue *et al*., 1995). With the exception of oxymetazoline, agonist E/[A] curves were displaced with high affinity by RS-17053 (data not shown). Furthermore, the high intrinsic activities obtained from experiments with amidephrine and A-61603 in particular, are strongly supportive of α_{1A}-adrenoceptor involvement (Minneman *et al*., 1994; Knepper *et al*., 1995).

An interesting finding from the present study is that the imidazoline, oxymetazoline, did not elicit increases in tension via an agonist effect at an α_1- or α_2-adrenoceptor population, but, apparently, via an effect on a 5-HT_2 receptor population shown previously to function in this tissue (Lachnit *et al*., 1996). Consequently, the ability of oxymetazoline to antagonise responses to A-61603 was evaluated. The estimate of affinity for oxymetazoline (pK_b=7.6) is in accordance with a pK_b estimate of 7.9 obtained in the rat perfused isolated kidney (an α_{1A}-adrenoceptor preparation; Blue *et al*., 1995). These data are also in agreement with pK_i values obtained from radioligand binding assays for the α_{1A}-, but not α_{1B}- or α_{1D}-adrenoceptor subtypes (Table 3; Minneman & Esbenshade, 1994).

It is intriguing that the α_1-adrenoceptor subtype mediating the response to A-61603 in rat caudal artery displays a pK_b value of 9.1 for prazosin. Such a value is slightly lower than seen in rat kidney (9.5; Blue *et al*., 1995) and vas deferens (9.3; Burt *et al*., 1995) at the α_{1A}-adrenoceptor but still does not approach the low estimates obtained for the α_{1L}-adrenoceptor (e.g.; 7.9, dog saphenous vein; Muramatsu *et al*., 1990a; 8.3, human prostate; Muramatsu *et al*., 1994) which form a basis for the so-called α_{1L}-adrenoceptor. Although the putative α_{1L}-adrenoceptor is also insensitive to CEC (Muramatsu *et al*., 1990a), as is the major contractile receptor found in the present study, the overriding pharmacological evidence fails to support its presence in rat caudal artery. The high affinities observed for RS-17053, SNAP 5089 and 5-methylurapidil are solely consistent with an α_{1A}-adrenoceptor (see Ford *et al*., 1996a). Thus, a marginally lower affinity estimate for prazosin may indicate that the α_{1A}-adrenoceptor displays tissue-dependent variability in affinity, perhaps arising from cell-specific influences on the conformation of the receptor protein (see Ford *et al*., 1996b).

The data from the present study are not totally consistent with a single receptor subtype mediating contractile responses to A-61603. A second site was revealed when E/[A] curves to A-61603 were displaced by more than 20 to 30 fold. Thus, Schild regressions (slopes significantly less than 1) obtained against responses to A-61603 for prazosin, RS 17053, 5-methylurapidil, and REC 15/2739 may indicate receptor heterogeneity in rat caudal artery. However, it is of interest that whereas slopes were slightly lower than 1 for some antagonists (see above), others gave Schild regressions of 1 (tamsulosin, SNAP 5089, HV 723). If an additional receptor population does contribute to contractile responses of A-61603, it is a population with higher affinity for these latter antagonist. Based on knowledge of this group of ligands, and currently defined α-adrenoceptors, it is difficult to deduce any population that would display these characteristics.

It has been shown previously that the caudal artery of rat contains functional α_2-adrenoceptors (Medgett & Langer, 1984; Atkinson *et al*., 1988). Although these post-junctional α_2-adrenoceptors may play a prominent vasoconstrictor role *in vivo* (Redfern *et al*., 1995), when assayed *in vitro*, they contract tissue only when basal tension is elevated by prior application of another spasmogen (Templeton *et al*., 1989; MacLean & McGrath, 1990). Furthermore, it has been shown recently that the postjunctional α_2-adrenoceptor functioning in rat caudal artery is of the α_{2C} subtype (Craig *et al*., 1995). The binding affinities (pK_i) of A-61603 at the recombinant α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors are 6.0, 4.6 and 5.4, respectively, but efficacy is very low at these subtypes (unpublished observations). Consequently, the involvement of α_2-adrenoceptors is probably very minor, but may have been sufficient to complicate the shape of Schild regressions in the current study, notably at E/[A] curve shifts greater than 20–30. Clearly, the finding that fluparoxan (3–30 μM) failed to shift further E/[A] curves to A-61603 that were already shifted by prazosin (1 μM) does not support this explanation.

In summary, caudal artery of rat contracts in response to NA via activation of at least two α_1-adrenoceptor subtypes. One of these subtypes displays the pharmacology of the α_{1A}-adrenoceptor, while the other remains to be defined. A-61603 allows for isolation of the α_{1A}-adrenoceptor component (approximate 30 fold window) and for quantitative pharmacological studies. In addition, data with RS-17053 and SNAP 5089 show that these selective α_{1A}-adrenoceptor antagonists, despite problematic physicochemical properties (i.e., high lipophilicity; especially for SNAP 5089), are useful pharmacological probes

that can be used at a low concentration in isolated tissue bath studies to characterize receptor populations. In light of these findings, the failure to observe high affinities with these agents in human lower urinary tract tissues under identical experimental conditions (Ford *et al*., 1996a) adds support to the α_{1L}-adrenoceptor characterization assigned to those tissues.

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