α_{1L}-Adrenoceptor mediation of smooth muscle contraction in rabbit bladder neck: a model for lower urinary tract tissues of man

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1   The α_1-adrenoceptor population mediating contractile responses to noradrenaline (NA) in smooth muscles of the bladder neck from rabbit (RBN) has been characterized by use of quantitative receptor pharmacology.

2   Experiments with several 'key' α_1-adrenoceptor antagonists of varying subtype selectivities (RS-17053, BMY 7378, indoramin, 5-methylurapidil, prazosin, REC 15/2739, SNAP 5089, terazosin, WB 4101, tamsulosin, (+)-cyclazosin and RS-100329) were conducted. Schild regression analyses yielded affinity (mean pK_b) estimates of 7.1, 6.2, 8.6, 8.6, 8.4, 9.3, 7.0, 7.4, 8.9, 10.0, 7.1 and 9.3, respectively, although deviations from unit Schild regression slope question the robustness of data for RS-17053 and SNAP 5089.

3   The nature of antagonism by these agents and the profile of affinity determinations generated together suggest that a single α_1-adrenoceptor subtype mediates contractile responses of RBN to NA. Additional studies with phenylephrine indicated also an agonist-independence of this profile. Pharmacologically, this profile was reminiscent of that described as 'α_{1L}'-adrenoceptor, which has been shown to mediate contractions of several tissues including lower urinary tract (LUT) tissues of man. Furthermore, a similarity was noticed between the 'α_{1L}'-adrenoceptor described here in RBN and the rabbit and human cloned α_{1a}-adrenoceptor (based on data from both whole cell radioligand binding at 37°C and [^{3}H]-inositol phosphates accumulation assays), characterizations of which have been published elsewhere.

4   In conclusion, the RBN appears to provide a predictive pharmacological assay for the study of NA-induced smooth muscle contraction in LUT tissues of man.

Keywords: Lower urinary tract; α_{1L}-adrenoceptor; α_{1A}-adrenoceptor; prazosin

Introduction

Current classification of α_1-adrenoceptors recognizes three subtypes, α_{1A}, α_{1B} and α_{1D} (Hieble *et al.*, 1995), which can be pharmacologically and molecularly distinguished. Numerous *in vitro* studies have demonstrated the presence of α_1-adrenoceptors in human lower urinary tract (LUT) tissues including prostatic smooth muscle (Hedlund *et al.*, 1985; Hieble *et al.*, 1985; Yamada *et al.*, 1987; Muramatsu *et al.*, 1994). It has been suggested (Hedlund *et al.*, 1985) that stimulation of α_1-adrenoceptors in the prostate of patients with benign prostatic hypertrophy can lead to the acute retention of urine, and clinical studies have confirmed that drugs blocking α_1-adrenoceptors can relieve outflow obstruction in these patients. The α_{1A}-adrenoceptor has been shown in radioligand binding and molecular biological studies to be the predominant α_1-subtype in these tissues (Price *et al.*, 1993; Faure *et al.*, 1994; Forray *et al.*, 1994). However, several studies have shown that the pharmacological profile of the α_1-adrenoceptor mediating contractile responses to noradrenaline (NA) in these tissues differs somewhat from the pharmacological profile in binding studies of the α_{1A}-adrenoceptor subtype (Ford *et al.*, 1997). These investigations have led some authors to suggest an alternative classification for this receptor based on its low affinity for prazosin ($pA_2 < 9$) (the 'α_{1L}'-adrenoceptor; Muramatsu *et al.*, 1990a,b).

The present investigations represent an attempt to develop a predictive screening assay system which would give clear, quantitative assessments of the activities and apparent affinities of α_1-adrenoceptor antagonists, reflective of their potential for activity in human LUT, by use of the rabbit bladder neck (RBN) as the comparative model

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Methods

Estimates of antagonist affinity (as pK_b) from functional (contractile) studies were made on isolated strips of RBN (male; New Zealand White; 3–3.5 kg; HRP, Inc., Denver, PA., U.S.A.). The bladder and urethra were removed from rabbits after CO_2 asphyxiation and placed in oxygenated Krebs solution (see below). Surrounding connective tissue and serosal layer were removed and longitudinal strips of bladder neck (1.5–2 mm wide, 5–7 mm long) were cut from the neck of the bladder, close to the proximal urethra (just above the prostate). Strips were resuspended in 10 ml organ baths (resting tension of 10 mN) for isometric measurement of changes in tension. Tension was recorded by means of Grass Instruments FT03 or Hugo Sachs K30 force-transducers connected to a Grass Model 7 polygraph or a Graphtex WR3310 linearcorder, respectively. All tissues were primed with NA (10 μM) after an appropriate equilibration period (15 min) and washed thoroughly to re-establish baseline tension before construction of two cumulative concentration-effect (E/[A]) curves to NA. Following construction of the first E/[A] curve, tissues were equilibrated with antagonist (or Krebs buffer in the case of time-controls) for 1–3 h.

For all studies, Krebs solution contained (in mM): NaCl 118.5, NaHCO$_3$ 25, KCl 4.8, MgSO$_4$ 1.2, KH$_2$PO$_4$ 1.2, CaCl$_2$ 2.5 and glucose 11, supplemented with 30 μM cocaine, 30 μM corticosterone, 100 μM ascorbate, 1 μM propranolol, 0.3 μM idazoxan and 10 μM indomethacin (in order to establish equilibrium conditions and to prevent involvement of β- and α_2-adrenoceptors, and cyclo-oxygenase products in responses to NA). All studies were performed at 35°C (in order to suppress basal myogenic activity) and solutions were gassed continuously with 5% CO_2 in O_2. In order to allow for optimal equilibrium conditions for the more lipophilic antagonists (e.g., SNAP 5089, RS-17053, REC 15/2739), strips of bladder neck were studied which were half the width of those described

above. It was found that larger strips yielded lower reproducibility of E/[A] curve shifts and affinity estimates for these antagonists, but not others (e.g., prazosin, WB 4101, 5-methylurapidil).

Data analysis

E/[A] curves were plotted by use of nonlinear iterative curve-fitting (Kaleidagraph software) to a form of the logistic equation for estimation of midpoint location parameter ($[A]_{50}$), such that $E = E_{max} \cdot [A]^{n_H}/([A]^{n_H} + [A]_{50}{}^{n_H})$ where E_{max} is the magnitude of the upper asymptote, and n_H is the Hill coefficient (defining the slope of the E/[A] relationship). Antagonist affinity estimates were obtained by construction of Schild regressions and were constrained to a slope of unity (if not statistically different) according to the equation: $pK_b = -\log[B] + \log(r-1)$, where [B] is the molar concentration of antagonist and r is the concentration-ratio of $[A]_{50}$ in the presence of antagonist divided by that obtained in the absence of antagonist. Estimates of r were corrected for variations in tissue sensitivity to agonist over time. The number of parameter determinations is denoted by *n*; all antagonists were tested on several tissues from at least 4 animals. Statistical analyses were performed by use of Statview II software ($P < 0.05$). Throughout the text, parameters are given as mean ± s.e.mean. Terms and equations are as recommended by the IUPHAR Committee on Receptor Nomenclature and Drug Classification (Jenkinson *et al.*, 1995).

Drugs and solutions

Compounds were obtained from the following sources: (±)-NA hydrochloride, (±)- and (−)-NA bitartrate, (−)-phenylephrine (PE) hydrochloride, (±)-propranolol hydrochloride, cocaine hydrochloride, corticosterone, indomethacin and prazosin hydrochloride: Sigma Chemical Co. (St. Louis, MO); WB 4101 (2 - (2,6 - dimethoxyphenoxyethyl) - aminomethyl-1,4-benzodioxane), idazoxan hydrochloride, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5] decane-7.9-dione dihydrochloride), and 5-methylurapidil: Research Biochemicals (Natick, MA); and (−)-tam-sulosin (YM 617), RS-17053 (N - [2 - (2 - cyclopropylmethoxyphenoxy)ethyl] - 5 - chloro - α,α - dimethyl -1H-indole-3-ethanaminehydrochloride), REC 15/2739 (SB 216469; 8-(3-[4-(2-methoxy-phenyl)-1-piper-

azinyl] -propylcarbamoyl)-3-methyl-4-oxo-2-phenyl-4H-1-ben-zopyran dihydrochloride), SNAP 5089 (2, 6-dimethyl-4-(4-nitrophenyl)1,4 -dihydropyridine - 3,5 - dicarboxylic acid - N[3 - (4,4 - diphenylpiperidine-1-yl)propyl]amide-methyl ester), (+) - cyclazosin and RS - 100329 (N-[(2-trifluoroethoxy)phenyl],N′-(3-thyminylpropyl)piperazine hydrochloride SDZ NVI 085 (3,4,4a5,10,10a - hexahydro - 6 - methoxy - 4 - methyl -9-methyl-thio -2H-naphth [2,3 -b] -1,4-oxazine hydro-chloride), A-61603-(± N - [5 - 4,5 - dihydro - 1 H - imidazol - 2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen - 1 - yl] methanesulphonamide hydro-bromide) and NS-49 ([R]-(-)-3′-(2-amino-1-hydroxyethyl) - 4″ - fluoromethane sulphonanilide hydrochloride): synthesized in the Chemistry Department, Neuro-biology Unit, Roche Bioscience (Palo Alto, CA). Stock solutions were prepared in distilled water or dimethylsulph-oxide (DMSO) on the day of study and diluted in appropriate buffer.

Results

NA produced concentration-dependent contractions of strips of RBN, with the second E/[A] curves, performed 1 or 3 h after
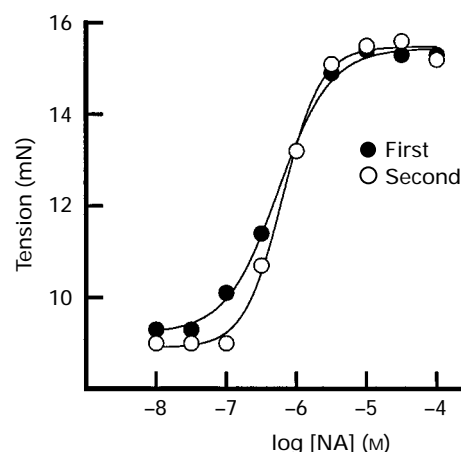

Figure 1 Effects of time on noradrenaline (NA)-induced contractile responses in isolated strips of rabbit bladder neck. Representative E/[A] curves in response to NA showing first curves and second curves (60 min after first).

Table 1 Affinity estimates (pK_b) for α_1-adrenoceptor antagonists in native tissues and recombinant receptors

Antagonist	Rabbit bladder neck (RBN)	Human LUT[b]	Rat caudal artery[a]	α_{1a}: human clone[c] (binding)	α_{1a}: human clone[d] ([³H-InsPs])	α_{1a}: rabbit clone[d] ([³H-InsPs])
Prazosin	8.4 ± 0.1	8.5 ± 0.1	9.2	8.4 ± 0.1	8.7 ± 0.1	8.7 ± 0.1
WB 4101	8.9 ± 0.0	8.9 ± 0.1	ND	8.9 ± 0.1	8.9 ± 0.1	8.6 ± 0.2
REC 15/2739	9.3 ± 0.1	9.2 ± 0.1	10.0	8.6 ± 0.2	9.4 ± 0.0[e]	ND
5-Methylurapidil	8.6 ± 0.1	8.2 ± 0.1	9.0	8.1 ± 0.1	8.2 ± 0.1	8.2 ± 0.1
Terazosin	7.4 ± 0.1	7.8 ± 0.0	ND	ND	ND	ND
Indoramin	8.6 ± 0.1[f]	8.5 ± 0.2	ND	8.3 ± 0.1	8.4 ± 0.1	8.3 ± 0.1
Tamsulosin	10.1 ± 0.1[f,g]	10.4 ± 0.1	11.2	10.0 ± 0.1	10.5 ± 0.1	10.3 ± 0.2
BMY 7378	6.2 ± 0.1[f]	6.4 ± 0.1	6.3	ND	ND	ND
RS-17053	7.12 ± 0.1	7.3 ± 0.1	9.2	7.9 ± 0.1	8.2 ± 0.1	7.9 ± 0.1
SNAP 5089	7.0 ± 0.1[f,g]	< 6.5[h]	9.5	ND	ND	ND
RS-100329	9.3 ± 0.1	9.0 ± 0.2[i]	ND	9.6 ± 0.1[i]	ND	ND
(+)-Cyclazosin	7.1 ± 0.1	ND	ND	7.5 ± 0.1[j]	7.6 ± 0.2	ND

[a]Lachnit *et al.* (1997; antagonists tested against A-61603). [b]Ford *et al.* (1996). [c]Williams *et al.* (1996). [d]Daniels *et al.* (1996). [e]Ford *et al.* (1997). [f]Suppression of maxima at higher concentrations of antagonist. [g]Schild slope significantly ≠ 1 ($P < 0.5$). [h]No significant shift observed at 0.3 μM. [i]Dr Timothy Williams, unpublished data. [j]bovine α_{1a} Giardinà *et al.* (1996). All values are mean ± s.e.mean for data from at least three determinations. ND = not determined.

the first curves, being not significantly different (Figure 1). All antagonists tested in RBN (Table 1) produced concentration-dependent parallel, rightward shifts in E/[A] curves to NA. However, indoramin, tamsulosin, BMY 7378, RS-17053 and SNAP 5089 did significantly suppress E/[A] curve maxima by at least 50%, although only at higher concentrations ($\geqslant 1$ μM, $\geqslant 1$ nM, $\geqslant 100$ μM, $\geqslant 3$ μM and $\geqslant 0.1$ μM, respectively).

Schild regression analyses were conducted with data from all antagonists. Antagonism with prazosin was concentration-dependent and the slope of the Schild regression analysis (0.98) was not significantly different from unity, consistent with attainment of conditions of equilibrium and simple competition at a single site (Figure 2a). This analysis yielded a pK_b of 8.4 ± 0.1 ($n = 19$). To confirm further whether antagonist
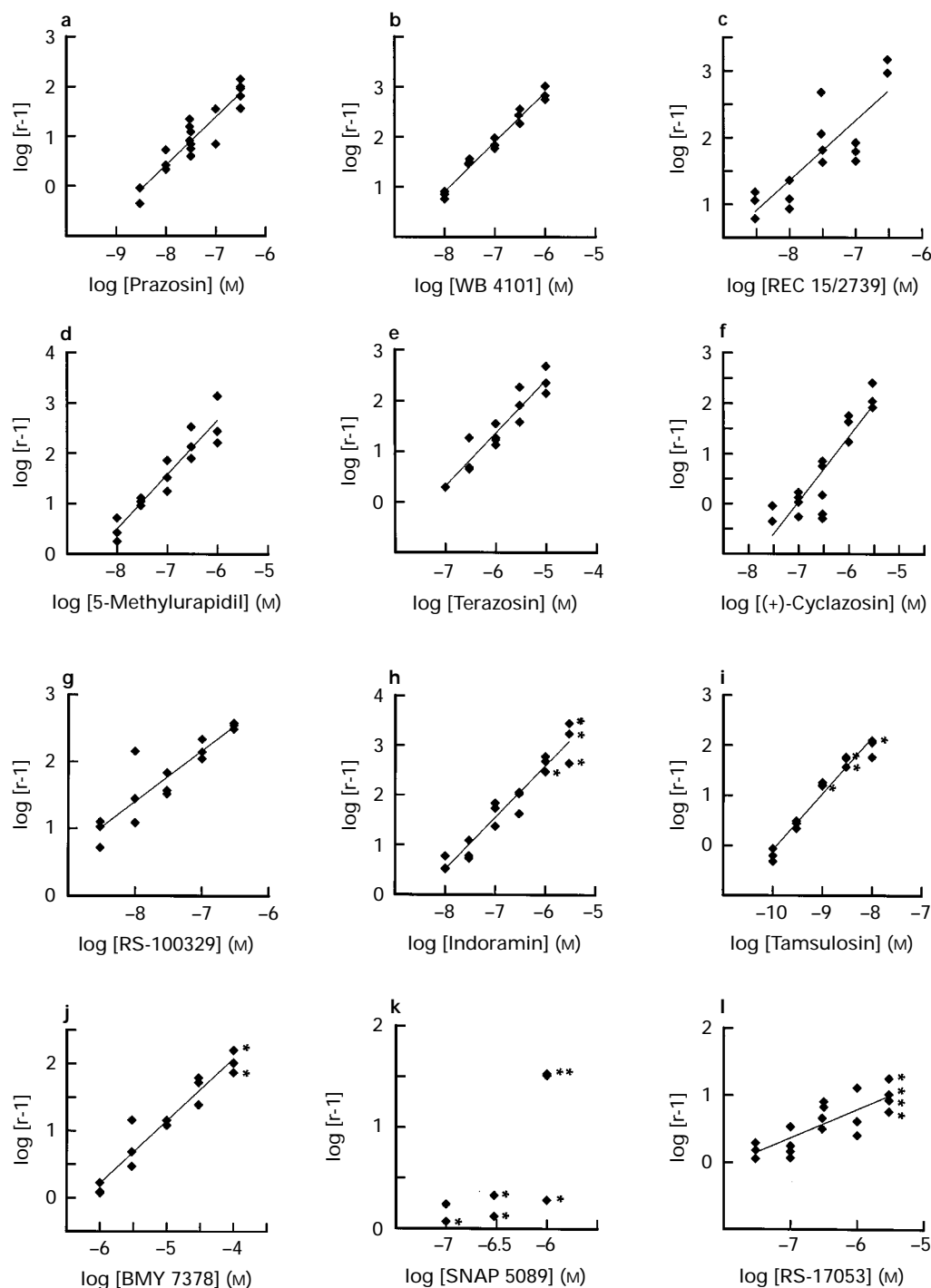

Figure 2 Effects of various α_1-adrenoceptor antagonists on NA-induced contractile responses in rabbit bladder neck: Schild regression analysis with concentration-ratios (r) estimated from individual strips of bladder neck (n = number of determinations) for (a) prazosin: Schild slope = 0.98 (95% CI: 0.80 − 1.15), $n = 19$; (b) WB 4101: Schild slope = 0.99 (95% CI: 0.91 − 1.08), $n = 15$; (c) REC 15/2739: Schild slope = 0.90 (95% CI: 0.56 − 1.26), $n = 15$; (d) 5-methylurapidil: Schild slope = 1.08 (95% CI: 0.87 − 1.30), $n = 15$; (e) terazosin: Schild slope = 1.05 (95% CI: 0.88 − 1.24), $n = 16$; (f) (+)-cyclazosin: Schild slope = 1.30 (95% CI: 0.92 − 1.66), $n = 17$; (g) RS-100329: Schild slope = 0.76 (95% CI: 0.55 − 1.0), $n = 15$; (h) indoramin: Schild slope = 1.04 (95% CI: 0.89 − 1.18), $n = 18$; (i) tamsulosin: Schild slope = 1.12, (95% CI: 0.98 − 1.26), $n = 15$; (j) BMY 7378: Schild slope = 0.93 (95% CI: 0.79 − 1.08), $n = 16$; and (k) SNAP 5089: Schild slope not able to be determined (significantly different from unity), $n = 7$; (l) RS-17053: Schild slope = 0.43 (significantly different from unity, 95% CI: 0.27 − 0.59), $n = 18$. Values where a suppression of maxima occurred are shown by asterisks and are excluded in estimation of mean affinities.

equilibrium had been achieved, prazosin was incubated with the tissues for 3 h before the construction of the second curves and, again, yielded a slope not significantly different from unity and a pK_b value of 8.6 ± 0.1. Schild regression analyses for all antagonists are shown in Figure 2. WB 4101 (Figure 2b), REC 15/2739 (Figure 2c). 5-methylurapidil (Figure 2d), terazosin (Figure 2e), (+)-cyclazosin (Figure 2f), and RS-100329 (Figure 2g) all produced Schild slopes not significantly different from unity; corresponding pK_b estimates (mean ± s.e.mean) and Schild regression slopes (with 95% CI) are shown in Table 1 and figure ligands, respectively. At higher concentrations, indoramin (Figure 2h), tamsulosin (Figure 2i), and BMY 7378 (Figure 2j) suppressed maximal response to the agonist. SNAP 5089 (Figure 2k) and RS-17053 (Figure 2l), unlike other antagonists studied, produced Schild slopes significantly different than unity in addition to a suppression of maxima at higher concentrations. Concentration-ratios displayed in Figure 2 from curves where response suppression was observed are indicated by asterisks. These data points, while not apparently deviating from the linear regression analyses, were excluded from affinity determinations. Tamsulosin also produced a Schild slope significantly greater than one as has also been found in other studies (Ford *et al.*, 1997). Consequently, the affinity estimates for SNAP 5089 and RS-17053 must be considered as preliminary.

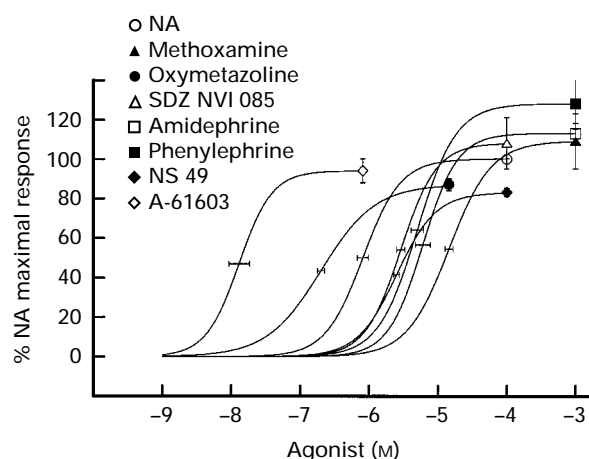Figure 3 shows the contractile response of strips of RBN to selected α_1-adrenoceptor agonists. Compared to NA, methoxamine, SDZ NVI 085, amidephrine and PE were full agonists, whereas oxymetazoline, NS 49 and A-61603 showed lower efficacy (Table 2). Table 2 also summarizes the results of these agonists with respect to potency ($p[A]_{50}$), intrinsic activity and Hill slope. To investigate agonist independence of antagonist affinity estimates, several antagonists were studied against responses to PE in this tissue; this yielded affinity estimates essentially identical to those obtained against NA (Table 1):

Figure 3 Cumulative E/[A] curves to selected α_1-adrenoceptor agonists on contractile responses in isolated strips of rabbit bladder neck. Mean E/[A] curves in response to NA, methoxamine, oxymetazoline, SDZ NVI 085, amidephrine, phenylephrine, NS 49 and A-61603. All data are expressed as a percentage of NA response maxima, obtained for each tissue. Mean parameters (with standard error bars) for $[A]_{50}$, E_{max} and n_H are shown.

Table 2 α_1-Adrenoceptor agonist potency in rabbit bladder neck

Agonist	n[1]	$p[A]_{50}$	Intrinsic activity	n_H
Noradrenaline	32	6.1 ± 0.1	1.0	1.6
Methoxamine	7	4.8 ± 0.1	1.1	1.4
Oxymetazoline	8	6.7 ± 0.1	0.9	1.1
SDZ NVI 085	4	5.5 ± 0.1	1.1	1.7
Amidephrine	8	5.2 ± 0.1	1.1	1.7
Phenylephrine	3	5.3 ± 0.1	1.3	1.5
NS 49	3	5.6 ± 0.0	0.8	1.5
A-61603	4	7.9 ± 0.2	0.9	1.8

[1]Number of determinations.

Figure 4 Effect of various α_1-adrenoceptor antagonists on PE-induced contractile responses in rabbit bladder neck: Schild regression analysis with concentration-ratios (r) estimated from individual strips of bladder neck (*n* = number of determinations) for (a) prazosin: Schild slope = 0.86 (95% CI: 0.70–1.02), *n* = 15; (b) indoramin: Schild slope = 1.0 (95% CI: 0.88–1.12), *n* = 10; (c) RS-100329: Schild slope = 1.0 (95% CI: 0.79–1.29), *n* = 14.

prazosin, 8.4 ± 0.1 (Figure 4a); REC 15/2739, 9.2 ± 0.1; terazosin, 7.7 ± 0.1; indoramin, 8.6 ± 0.0 (Figure 4b); tamsulosin, 9.7 ± 0.1; BMY 7378, 6.5 ± 0.2; RS-17035, 7.0 ± 0.1; SNAP 5089, 7.4 ± 0.2; RS-100329, 9.3 ± 0.1 (Figure 4c). When PE was used as the agonist, suppression of maximal response (by at least 50%) was seen with SNAP 5089 ($\geqslant 100$ nM), RS-17053 ($\geqslant 3$ μM) and tamsulosin ($\geqslant 10$ nM). However, in contrast to NA responses, this phenomenon was not seen with indoramin (to 100 nM) or BMY 7378 (to 3 μM).

Discussion

In a recent study of second messenger responses of cloned α_{1a}-adrenoceptors from human, rat, and rabbit (all expressed in CHO-K1 cells), Daniels *et al.* (1996) showed that several antagonists, including prazosin, RS-17053, WB 4101 and 5-methylurapidil failed to display the typical subnanomolar affinities believed to be 'characteristic' of the α_{1A}-subtype, while expected high affinities were still displayed by others, including, tamsulosin, REC 15/2739 and indoramin. However,

this profile of antagonist affinity values had previously been found to be consistent with that obtained from functional (contractile) studies of LUT tissues of man, at the so-called 'α_{1L}'-adrenoceptor, and was distinctly different from estimates observed in the caudal artery (Lachnit *et al.*, 1997) or perfused kidney (Blue *et al.*, 1996) of rat (each of which display 'classical' α_{1A}-adrenoceptor characteristics). Furthermore, recent studies conducted by Williams *et al.* (1996) have extended the observations of apparent α_{1A}- : α_{1L}-adrenoceptor equivalence, by revealing that in whole-cell binding studies conducted under physiological conditions (37°C, isotonic media), the α_{1a}-adrenoceptor gene product displays distinct pharmacological recognition state consistent with the 'α_{1L}'-subtype, and different from that observed in homogenates at room temperature. Thus, depending upon the prevailing experimental conditions, the α_{1a}-adrenoceptor gene product can be observed to display either α_{1A}- or α_{1L}-adrenoceptor pharmacology (see Ford *et al.*, 1997).

Table 1 shows antagonist affinity estimates from the present study and compares these with data published previously for the same compounds from studies of contraction of human
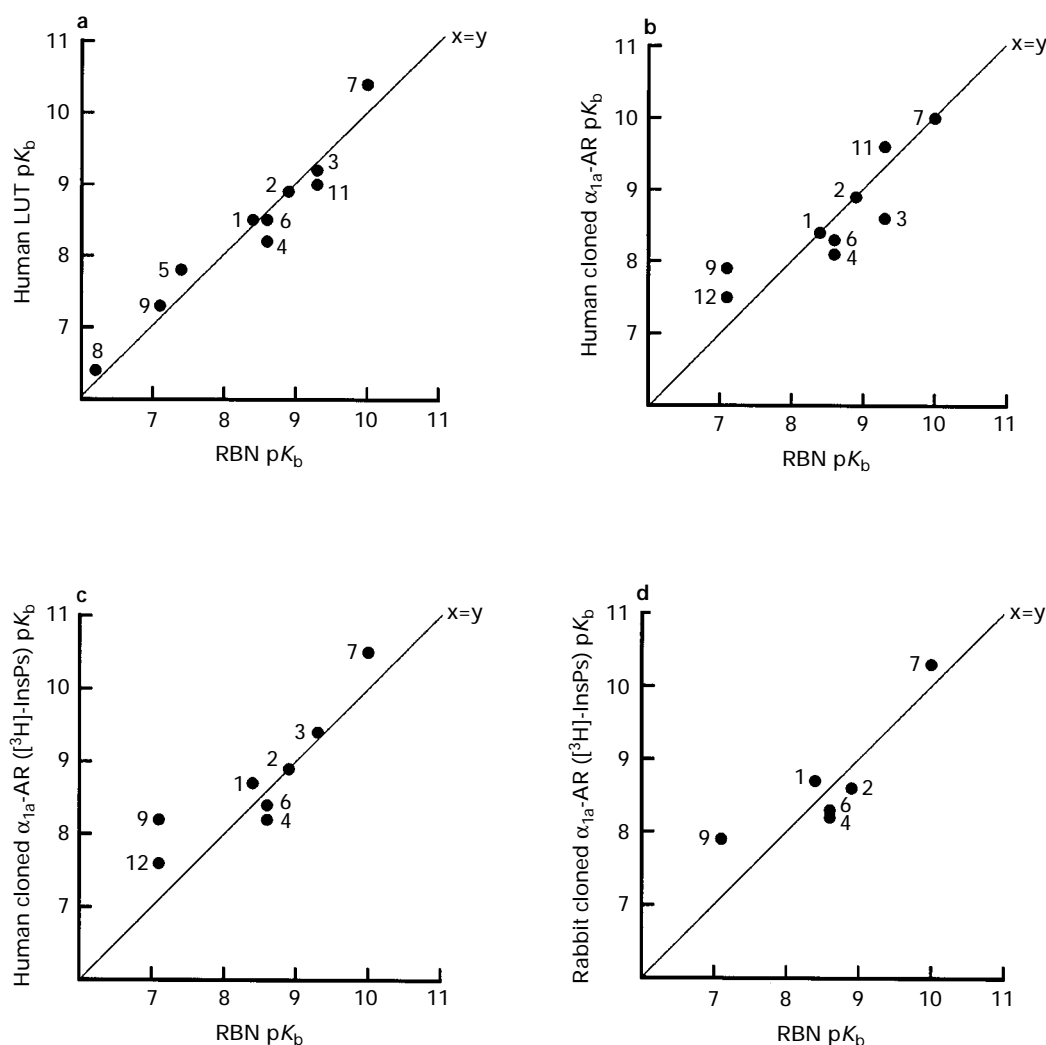

Figure 5 Correlation plots showing the relationship of affinity estimates from *in vitro* functional assays of RBN tissues for selected antagonists compared with (a) human lower urinary tract (LUT) (mean $\Sigma(y-x)^2 = 0.07$), (b) human cloned α_{1a}-adrenoceptors (37°C binding) (mean $\Sigma(y-x)^2 = 0.19$), (c) human cloned α_{1a}-adrenoceptors ([^{3}H]-InsPs accumulation assay) (mean $\Sigma(y-x)^2 = 0.25$), and (d) rabbit cloned α_{1a}-adrenoceptors ([^{3}H]-InsPs accumulation assay) (mean $\Sigma(y-x)^2 = 0.19$). In each plot, the solid line indicates the line of identity ($y = x$). Mean $\Sigma(y-x)^2$ is the meaned sum of squares of differences in affinity estimates for each plot, and describes, in relative terms, how 'different' the two data sets are. Data are from Table 1: (1) prazosin; (2) WB 4101; (3) REC 15/2739; (4) 5-methylurapidil; (5) terazosin; (6) indoramin; (7) tamsulosin; (8) BMY 7378; (9) RS-17053; (11) RS-100329; (12) (+)-cyclazosin.
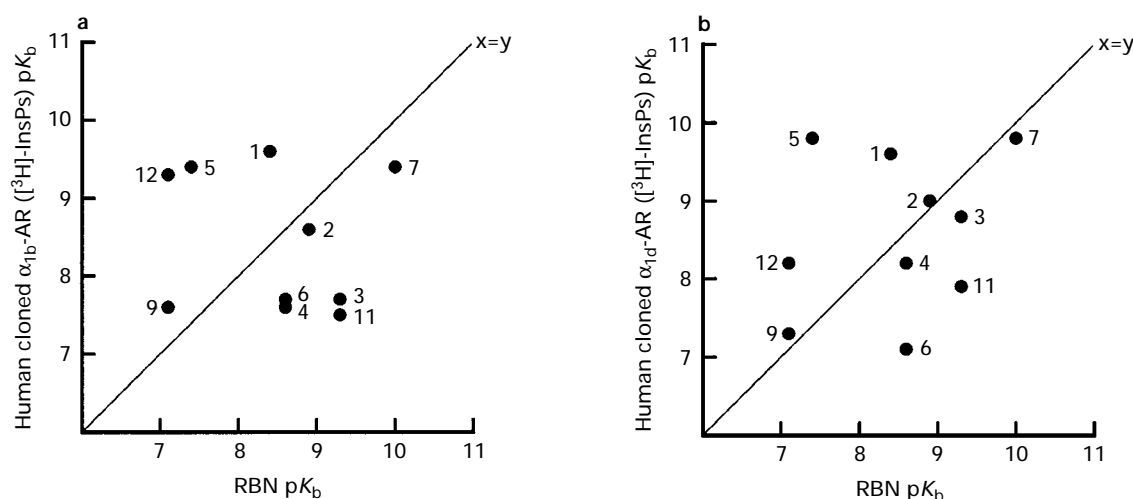
Figure 6 Correlation plots showing the relationship of affinity estimates from *in vitro* functional assays of rabbit bladder neck tissues for selected antagonists compared with (a) human cloned α_{1b}-adrenoceptors ([³H]-InsPs accumulation assay; Ford *et al.*, 1997) (mean $\Sigma(y-x)^2 = 1.86$) and (b) human cloned α_{1d}-adrenoceptors ([³H]-InsPs accumulation assay; Ford *et al.*, 1997) (mean $\Sigma(y-x)^2 = 1.31$). In each plot, the solid line indicates the line of identity ($y=x$). Mean $\Sigma(y-x)^2$ is the meaned sum of squares of differences in affinity estimates for each plot, and describes, in relative terms, how 'different' the two data sets are. Data for; (1) prazosin; (2) WB 4101; (3) REC 15/2739; (4) 5-methylurapidil; (5) terazosin; (6) indoramin; (7) tamsulosin; (9) RS-17053; (11) RS-100329; (12) (+)-cyclazosin.

LUT tissues (Ford *et al.*, 1996), whole-cell radioligand binding at human cloned α_{1a}-adrenoceptors (Williams *et al.*, 1996), and second messenger generation in CHO-K1 cells stably expressing human and rabbit cloned α_{1a}-adrenoceptors ([³H]-inositol phosphates (InsPs) accumulation assays; Daniels *et al.*, 1996). As detailed in Results, many of the antagonists tested in this study behaved in a manner that is acknowledged to be consistent with (though not necessarily indicative of) simple, reversible competitive antagonism at a singular receptor population, under conditions of apparent equilibrium. Accordingly, the agonist-independence (for prazosin, indoramin and RS-100329) and time-independence (prazosin) of selected affinity estimates were also demonstrated (Figures 1 and 3). In some cases, particularly when pharmacological tools were used that are less physicochemically attractive for equilibrium studies (see Ford *et al.*, 1996; 1997), deviations from simple reversible competition were observed, reflected by steep Schild regressions and, occasionally, degrees of insurmountability at higher antagonist concentrations (values where a suppression of maxima occurred are shown by asterisks in Figure 2 and are excluded in estimation of mean affinities for Table 1). Although no explanatory mechanisms are obvious to account for such deviations, previous studies have encountered and demonstrated similar behaviour with the same antagonists (e.g., SNAP 5089, RS-17053, tamsulosin; Ford *et al.*, 1996; 1997; Noble *et al.*, 1997; Lachnit *et al.*, 1997). While these latter studies offer little evidence for lack of competition, evidence has been presented for loss of low concentrations of antagonist due to non-specific adsorption onto plastics and glass or degradation (feasibly introducing steep Schild regressions), together with low rates of dissociation and poor surmountability (see Lachnit *et al.*, 1997). Indeed, as these shortcomings in the use of certain antagonists were also observed in human LUT tissues (Ford *et al.*, 1997) the similarity with RBN tissues is further underlined.

Figure 5 illustrates and compares the relationships found between affinity estimate profiles displayed in Table 1. In each figure, the line of identity ($y=x$) was drawn (solid line). In Figure 5a, it can be clearly seen that the relationship between affinity estimates for human LUT and RBN is good, with all points lying close to the line of identity, with a low mean sum

of squares of affinity differences (mean $\Sigma(y-x)^2 = 0.07$) supporting this equality. The RBN determinations also correlate well with those from human cloned α_{1a}-adrenoceptors (37°C whole-cell binding; Figure 5b), as well as functional second messenger studies from human cloned α_{1a}-adrenoceptors ([³H]-InsPs accumulation; Figure 5c) and rabbit cloned α_{1a}-adrenoceptors ([³H]-InsPs; Figure 5d), where mean sums of squares of affinity differences observed were 0.19, 0.25, and 0.19, respectively. In contrast, Figure 6a and b shows correlations comparing data from RBN with human cloned α_{1b}- and α_{1d}-adrenoceptors ([³H]-InsPs; Ford *et al.*, 1997). Both correlations yield greater mean sums of squares of affinity differences which indicate that these α_1-adrenoceptors do not resemble the functional receptor population in RBN.

To extend the observations with the antagonists, studies were conducted with a variety of α_1-agonists (Figure 3, Table 2). The receptor population functioning in RBN was activated efficiently by the panel of agonists used, with all the agonists selected displaying full or close to full agonist activity. These data, in particular those with amidephrine and its analogue, NS-49, methoxamine and its analogue SDZ NVI 085, and the imidazolines, oxymetazoline and A-61603, are consistent with activation of α_{1A}- or α_{1L}-adrenoceptors but clearly not with activation of α_{1B}- and α_{1D}-adrenoceptors (see Lachnit *et al.*, 1997; Ford *et al.*, 1997). This is also in agreement with data from experiments on transfected CHO-K1 cells, obtained by Minneman *et al.* (1994), which also showed the apparent high efficacy of amidephrine, oxymetazoline, NS-49 and methoxamine and their weak activity at the other subtypes. Previous data from our laboratory have indicated that α_{1A}- and α_{1L}-adrenoceptors cannot be distinguished pharmacologically on the basis of agonist data (see Ford *et al.*, 1997), thus the antagonist data arising from this study carry considerably greater significance in terms of α_1-adrenoceptor characterization. The α_1-adrenoceptor in human LUT and RBN displays the moderately lower affinity for prazosin and other antagonists (e.g. RS-17053) that have given rise to the appellation 'α_{1L}'-adrenoceptor. The pharmacological profile of this receptor has emerged gradually and includes low affinity for prazosin (Holck *et al.*, 1983; Flavahan & VanHoutte, 1986) and relatively low affinities for WB 4101, 5-methylurapidil

(Muramatsu, 1992), RS-17053 and the dihydropyridines, S-niguldipine and SNAP 5089 (Ford *et al.*, 1996), especially when compared with affinity estimates from the classically defined α_{1A}-adrenoceptors. Similarly, from the present study, and as found recently by Hopkins and Kenny (1997) and Leonardi *et al.* (1997), it is clear that the α_1-adrenoceptor in the RBN does not conform by antagonist pharmacological profile, with classically defined α_{1a}-, α_{1b}- or α_{1d}-adrenoceptors, as reflected by homogenate binding data for these clones, or to the functionally-defined 'classical' α_{1A}-, α_{1B}-, or α_{1D}-adrenoceptors, all of which display high affinity for prazosin ('α_{1H}'). To date, no antagonists have been demonstrated categorically to recognize the putative α_{1L}-adrenoceptor with higher affinity than that displayed at the α_{1A}-adrenoceptor, although it is clear that certain α_{1A}-selective antagonists do display equivalent high affinity for the α_{1L}-adrenoceptor, including RS-100329 (this study) and JTH-601 (Maramatsu *et al.*, 1996). Obviously, there is scope for improvement in characterization of the α_{1L}-adrenoceptor should truly selective compounds be discovered.

Whether the receptor population defined in RBN, which is clearly analogous to the putative α_{1L}-adrenoceptor, should retain this operationally based nomenclature remains to be determined. If the α_{1L}-adrenoceptor really exists as a distinct pharmacological recognition state of the α_{1a}-adrenoceptor (Ford *et al.*, 1997), as would appear to be the case, then it is essential that a contemporary classification and nomenclature system reflects this new finding.

In summary, these data indicate that contractile responses to NA in bladder neck strips from rabbit are mediated by an apparently homogenous α_1-adrenoceptor population which displays pharmacological properties consistent with those observed for NA-induced contraction of human LUT tissues. Consequently, this preparation can be used as a highly predictive assay upon which the target activity of novel uroselective α_1-adrenoceptor antagonists can be assessed.

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