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Evaluation of the pharmacological selectivity profile of α_1 adrenoceptor antagonists at prostatic α_1 adrenoceptors: binding, functional and *in vivo* studies

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1 The profile of a range of α_1 adrenoceptor antagonists was determined *in vitro* against cloned human α_{1A}, α_{1B} and α_{1D} adrenoceptors and against noradrenaline-mediated contractions of rat aorta and human prostate. The *in vivo* profile of compounds was determined in an anaesthetized dog model which allowed the simultaneous assessment of antagonist potency against phenylephrine-mediated increases in blood pressure and prostatic pressure.

2 The quinazoline antagonists, prazosin, doxazosin and alfuzosin displayed high affinity but were non selective for the three cloned human α_1 adrenoceptors. Indoramin and SNAP 1069 showed selectivity for α_{1A} and α_{1B} adrenoceptors relative to the α_{1D} subtype. Rec 15/2739, WB 4101, SL 89,0591, (+)- and (−)-tamsulosin showed selectivity for α_{1A} and α_{1D} adrenoceptors relative to the α_{1B} subtype. RS 17053 showed high affinity and selectivity for α_{1A} adrenoceptors (pK_i 8.6) relative to α_{1B} ($pK_i = 7.3$) and α_{1D} ($pK_i = 7.1$) subtypes.

3 (+)-Tamsulosin, (−)-tamsulosin, SL 89,0591, Rec 15/2739, SNAP 1069 and RS 17053 appeared to act as competitive antagonists of noradrenaline-mediated contractions of rat aorta yielding pA_2 affinity estimates which were similar to binding affinities at cloned human α_{1D} adrenoceptors. The following rank order was obtained: prazosin = (−)-tamsulosin > doxazosin > SL 89,0591 = (+)-tamsulosin > Rec 15/2739 > RS 17053 = SNAP 1069.

4 (−)-Tamsulosin was a very potent, insurmountable antagonist of noradrenaline-mediated contractions of human prostate, yielding an approximate pA_2 estimate of 9.8 at 1 nM. The corresponding (+)-enantiomer was 30 fold weaker. SL 89,0591, SNAP 1069 and Rec 15/2739 yielded pA_2 estimates which compared well with their α_{1A} binding affinities. The affinity estimate for prazosin on human prostate was lower than the corresponding binding affinity determined at α_{1A} adrenoceptors and RS 17053 was a very weak antagonist on human prostate ($pA_2 = 6.0$) relative to the high affinity ($pK_i = 8.6$) determined at cloned human α_{1A} adrenoceptors.

5 In the anaesthetized dog, *in vivo* pseudo 'pA_2' values showed that doxazosin, (+)- and (−)-tamsulosin inhibited phenylephrine-induced increases in prostatic and blood pressure with similar affinity, implying that these agents show little or no selectivity for prostatic responses in this model. SL 89,0591 and SNAP 1069 were moderately selective (3 and 6 fold respectively) for prostatic pressure relative to blood pressure. Rec 15/2739 was a more potent antagonist of phenylephrine-mediated increases in prostatic pressure ('pA_2' = 8.74) compared to blood pressure ('pA_2' = 7.51).

6 Data in this study suggest that the α_1 adrenoceptor mediating noradrenaline-induced contractions of human prostate, whilst having some of the characteristics of an α_{1A} adrenoceptor, cannot be satisfactorily aligned with cloned α_{1A}, α_{1B} or α_{1D} adrenoceptors. In addition, studies in the anaesthetized dog have shown that agents having high affinity and selectivity for prostatic α_1 adrenoceptors, particularly over the α_{1D} subtype, appear to inhibit phenylephrine-induced increases in prostatic pressure selectively compared to blood pressure.

Keywords: α_1 Adrenoceptors; 'human prostate'; adrenoceptor antagonists; anaesthetized dog

Introduction

Benign prostatic hyperplasia (BPH) is a hormone-dependent neoplasm in which progressive enlargement of the prostate leads to bladder outlet obstruction which causes a disturbance in normal urinary outflow, urinary retention and associated irritative symptoms (McNeal, 1990). In symptomatic BPH, two components are associated with urethral obstruction; a static component related to prostatic tissue mass, and a dynamic component related to the sympathetic tone of prostatic and urethral smooth muscle. A dense network of adrenergic fibres innervates the prostate, and α_1 adrenoceptors have been identified in human prostatic tissue by autoradiography and radioligand binding studies (Chapple *et al.*, 1989; Lepor *et al.*, 1993; Kobayashi *et al.*, 1993). It is also well established that noradrenaline and phenylephrine mediate contractile responses of prostatic tissue *in vitro* almost exclusively through an interaction with α_1 adrenoceptors (Hieble *et al.*, 1985).

α_1 Adrenoceptors are not a homogeneous entity and pharmacological studies have consistently demonstrated heterogeneity in native tissues (Ruffolo *et al.*, 1991). These findings are supported by molecular cloning studies which have identified three α_1 adrenoceptor subtypes, reflected by current nomenclature which recognizes α_{1A}, α_{1B} and α_{1D} subtypes (Ford *et al.*, 1994; Hieble *et al.*, 1995; Michel *et al.*, 1995). This classification scheme, however, accounts only for pharmacologically defined receptors and corresponding cloned receptors which display high affinity for prazosin. In contrast, binding and functional studies in a number of smooth muscle preparations have consistently identified α_1 adrenoceptors at which prazosin

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exhibits considerably lower affinity. An alternative classification scheme was originally proposed by Flavahan & Vanhoutte (1986) and more recently adopted by Muramatsu *et al.* (1990) which recognizes α_1 adrenoceptors with relatively high affinity for prazosin (α_{1H}; $pA_2/pK_D > 9.0$, encompassing all subtypes in the α_{1A}, α_{1B} and α_{1D} classification) and receptors with low affinity for prazosin (α_{1L}).

Using RNA extracted from human prostate, RNase protection assays have shown that the α_{1A} adrenoceptor mRNA represents more than 70% of the total α_1 mRNA in human prostate and *in situ* hybridization experiments have shown that α_{1A} mRNA localizes to the stromal compartment, consistent with radioligand binding to prostatic tissue sections using receptor autoradiography (Price *et al.*, 1993; Kobayashi *et al.*, 1993). The pharmacological characteristics of human prostatic α_1 adrenoceptors have been determined by a number of groups in which the majority of studies suggest a predominant role for α_{1A} adrenoceptors (Kenny *et al.*, 1995b). Comparative functional affinity estimates for a range of α_1 antagonists on human prostate have been found to correlate highly with binding affinities at the cloned α_{1A} adrenoceptor (Forray *et al.*, 1994; Marshall *et al.*, 1995). Similarly, the affinity of α_1 adrenoceptor antagonists determined against [^{125}I]-HEAT in human and canine prostatic tissue homogenates closely resembles affinities at cloned human α_{1A} adrenoceptors, but not with α_{1B} or α_{1D} subtypes (Goetz *et al.*, 1994). However, this conclusion is not uniformly accepted since the relatively low functional affinity exhibited by prazosin on human and canine prostate *in vitro* suggests that the receptor is more characteristic of an α_{1L} subtype (Muramatsu *et al.*, 1994).

An assessment of prostatic selectivity exhibited by α_1 adrenoceptor antagonists has been addressed in several studies (Lefevre-Borg *et al.*, 1993; Testa *et al.*, 1994; Kenny *et al.*, 1994; 1995b). Whilst several compounds such as alfuzosin, 5-methyl-urapidil and Rec 15/2739 have been claimed to lower prostatic tone selectively relative to blood pressure *in vivo*, conclusions have not been consistent between studies. Furthermore, in some of these studies no attempt has been made to correlate selectivity for the prostate *in vivo* with selectivity for any of the subtypes *in vitro*. The published data indicate that compounds having no subtype selectivity for any of the α_1 adrenoceptor subtypes *in vitro* exhibit a balanced profile *in vivo* towards prostatic and blood pressure (Kenny *et al.*, 1994; Testa *et al.*, 1994).

In this paper, we describe the profile of a number of compounds at α_1 adrenoceptor subtypes *in vitro* and assess the relevance of this selectivity in an anaesthetized canine model which allows the simultaneous assessment of prostatic pressure and blood pressure.

Methods

[^{3}H]-prazosin binding to cloned human α_1 adrenoceptors

Cloning of human α_1 adrenoceptor cDNAs and stable transfection into rat-1 fibroblasts was carried out as previously described (Schwinn *et al.*, 1995). Radioligand binding experiments were performed with membranes prepared from rat 1 fibroblast cells expressing individual α_1 subtypes essentially as previously described (Kenny *et al.*, 1994). 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 48000 *g*) and resuspended in fresh buffer before storage at −70°C. Cell membranes were stored at 0.5 mg ml^{-1} protein. Displacement of [^{3}H]-prazosin binding (0.2 nM) was measured in diluted membranes (2–10 μg fibroblast protein) in 50 mM Tris buffer (pH 7.5) in a final assay volume of 500 μl. At least 12 concentrations of competing compound were used for each determination. Non specific binding were determined in the presence of 1 μM phentolamine. 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. 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 119, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, 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 (1 μM) and washed over a 30 min period. Isometric contractions were obtained in response to cumulative additions of (−)-noradrenaline in all tissues to obtain control curves. A further curve was then generated 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).

Contractile responses of human prostate

Prostatic tissue was cut into longitudinal strips (approximately 3 × 2 × 10 mm) and suspended in organ baths under a resting tension of 1 g in Krebs Ringer bicarbonate of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11 and gassed with 95% O_2/5% CO_2. The solution also contained 10 μM cocaine and 10 μM corticosterone. Tissues were exposed to a sensitizing dose of (−)-noradrenaline (100 μM) and washed over a 45 min period. Isometric contractions were obtained in response to cumulative additions of (−)-noradrenaline to obtain control curves in all tissues. A further curve was then generated in the presence or absence of antagonist (incubated for 2 h). Due to limited amounts of human prostatic tissue available, antagonist affinity estimates (pA_2) were determined with a single concentration of competing antagonist, $pA_2 = -\log [A]/(DR-1)$ where the dose ratio (DR), relative to corresponding controls, was produced by a single concentration of antagonist [A], assuming competitive antagonism and Schild regression close to unity.

Anaesthetized dog model of prostatic pressure and blood pressure

The effect of α_1 antagonists on prostatic urethral pressure and blood pressure was determined essentially as previously described (Kenny *et al.*, 1994). Mature male beagles (12–15 kg body weight) were anaesthetized with sodium pentobarbitone (30–50 mg kg^{-1}, i.v.) and a tracheal cannula was inserted. Subsequent anaesthesia was maintained by pentobarbitone infusion. The animals were respired with air using a Bird Mk8 respirator (Bird Corp., Palm Springs, CA, U.S.A.) adjusted to maintain blood gases in the range Po_2 90–110 mmHg, Pco_2 35–45 mmHg, pH 7.35–7.45. Body temperature was maintained at 36–37.5°C with a heated operating table. Catheters were placed in the left femoral artery for recording blood pressure and in the left femoral vein for compound administration. Heart rate was recorded via the lead II E.C.G. A laparotomy was performed to cannulate both ureters to prevent change of fluid volume within the bladder. A 7F cardiac catheter (with a 1.5 ml capacity balloon tip) was inserted into the bladder via the urethra. The balloon was filled with air and the catheter withdrawn until the balloon became lodged in the prostate, which was confirmed by digital pressure. Balloon pressure was recorded via

a Druck transducer. Prostatic pressure and haemodynamic parameters were made on a Grass Polygraph (Grass Instruments, Quincy, Mass, U.S.A.) and the data measured online with a Motorola 68000-based microcomputer system (Motorola Inc., Temple, AZ, U.S.A.). Compounds were made up in PEG 300 and administered i.v. through a catheter in the femoral vein. Responses to phenylephrine (1–16 μg kg^{-1}, i.v. in saline) were obtained to generate control dose-response curves (two control curves for each experiment). Compounds were administered (in terms of compound base) at 10–300 μg kg^{-1}, i.v. 5 min before construction of phenylephrine curves (constructed up to a maximum dose of 128 μg kg^{-1} in the presence of test compound).

Due to α_1-related dysrhythmic properties of phenylephrine, absolute maximal responses were not obtained but were taken as 10% greater than the control response obtained with 16 μg kg^{-1} phenylephrine. Drug concentrations were calculated on the basis of molar weight of compound kg^{-1} body weight thus allowing a 'pseudo pA_2' calculation by Schild analysis using dose-ratios derived from shifts in the phenylephrine dose-response curves. We have shown in previous studies that little or no effect is seen with time-matched vehicle controls (Kenny *et al.*, 1994).

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.). 5-methyl-urapidil, BMY 7378 (8-[2-[4-(2-meth-oxyphenyl)-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, (+)- and (−)-tamsulosin, SL 890591 (2-{3-[4-(5-chloro-2-methoxyphenyl)-piperazin-1-yl]-propylamino}-pyrimidine-4-carboxylic acid amide), RS 17053 ([2-(5-chloro-1H-indol-3-yl)-1,1-dimethyl-ethyl]-[2-(2-cyclopro-pylmethoxy-phenoxy)- ethyl]-amine), Rec 15/2739 (3-methyl-4-oxo-2-phenyl-4H-chromene-8-carboxylic acid {3-[4-(2-methoxyphenyl)-piperazin-1-yl]-propyl}-amide) 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 dimethylsulphoxide (DMSO) (with minimal lactic acid) at 1 mM and subsequent dilutions made in distilled H_2O or assay buffer.

Results

Antagonist affinities at cloned human α_1 adrenoceptors

The pharmacological profile of compounds at cloned 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 the human α_{1D} subtype was 1 pmol mg^{-1} but was 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 0.34±0.05, 0.20±0.03 and 0.22±0.05 for human α_{1A}, α_{1B} and α_{1D} adrenoceptors respectively.

Displacement affinities (pK_i) for compounds tested are shown in Table 1. Prazosin, doxazosin, and alfuzosin displayed high and similar affinity for all three cloned human α_1 adrenoceptors. Indoramin and SNAP 1069 showed selectivity for α_{1A} and α_{1B} adrenoceptors relative to the α_{1D} subtype. Rec 15/2739, WB 4101, SL 89,0591, (+)- and (−)-tamsulosin showed selectivity for α_{1A} and α_{1D} adrenoceptors relative to the α_{1B} subtype. RS 17053 and showed high affinity and selectivity for α_{1A} adrenoceptors.

Antagonism of contractile responses in vitro

Rat aorta Noradrenaline produced concentration-dependent contractions of rat aorta with a pEC_{50} of 8.0±0.04 ($n=20$). (+)-Tamsulosin, (−)-tamsulosin, SL 89,0591, Rec 15/2739, SNAP 1069 and RS 17053 produced surmountable concentration-dependent rightward shifts of noradrenaline concentration-response curves, with resulting Schild plots for competing antagonists having regression slopes close to unity suggesting competitive antagonism. (−)-Tamsulosin was considerably more potent ($pA_2=9.7$) than the corresponding (+)-enantiomer ($pA_2=8.2$) (Figure 1). Affinity estimates for compounds on rat aorta (pA_2 values; Table 2) were similar to binding affinities at cloned human α_{1D} adrenoceptors, at which the following rank order was obtained: Prazosin = (−)-tamsulosin > doxazosin > SL 89,0591 = (+)-tamsulosin > Rec 15/2739 > RS 17053 = SNAP 1069.

Human prostate Noradrenaline produced concentration-dependent isometric contractions of human prostate, yielding a pEC_{50} of 5.7±0.05 ($n=20$). In the presence of α_1 adrenoceptor antagonists, concentration-response curves were shifted to the right yielding pA_2 affinity estimates shown in Table 2. Consistent with the profile at cloned α_{1A} adrenoceptors, (−)-tamsulosin was a very potent antagonist of noradrenaline-mediated contractions of human prostate, although responses to noradrenaline were insurmountable in the presence of increasing concentrations of this compound. At the lowest concentration tested, 1 nM, a pA_2 value of 9.8±0.11 was estimated. On this basis it was 30 fold more potent than the corresponding (+)-enantiomer (Figure 2). Affinity estimates for several other compounds examined were similar to binding affinities at the cloned α_{1A} adrenoceptor (Table 1; Figure 3). The affinity estimate for prazosin on human prostate was lower than the corresponding binding affinity determined at α_{1A} adrenoceptors and RS 17053 was a very weak antagonist on human prostate ($pA_2=6.0$) relative to the high affinity ($pK_i=8.6$) determined at cloned human α_{1A} adrenoceptors.

Effects of α_1 antagonists in the anaesthetized dog in vivo

Phenylephrine (1–16 μg kg^{-1}) produced dose-dependent increases in both prostatic and blood pressure responses which were antagonized in the presence of α_1 adrenoceptor antago-

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

Compound	pK_i Human α_{1A}	Human α_{1B}	Human α_{1D}
*Prazosin	9.7±0.20	9.6±0.14	9.5±0.10
*Doxazosin	8.5±0.20	9.0±0.20	8.4±0.12
*Alfuzosin	8.0±0.20	8.0±0.13	8.5±0.07
*SNAP 1069	7.8±0.19	7.6±0.18	6.8±0.20
*Spiperone	7.6±0.12	8.8±0.16	8.1±0.03
*Indoramin	8.3±0.03	8.0±0.12	7.3±0.15
(+)-Tamsulosin	8.4±0.06	7.0±0.08	8.1±0.04
(−)-Tamsulosin	9.7±0.06	8.9±0.06	9.8±0.09
Rec 15/2739	9.0±0.07	7.5±0.06	8.6±0.07
SL 89,0591	8.6±0.08	7.9±0.08	8.6±0.03
RS 17053	8.6±0.09	7.3±0.09	7.1±0.09

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. *Data for these compounds have previously been reported and are included for comparative purposes (Kenny *et al.*, 1995a).

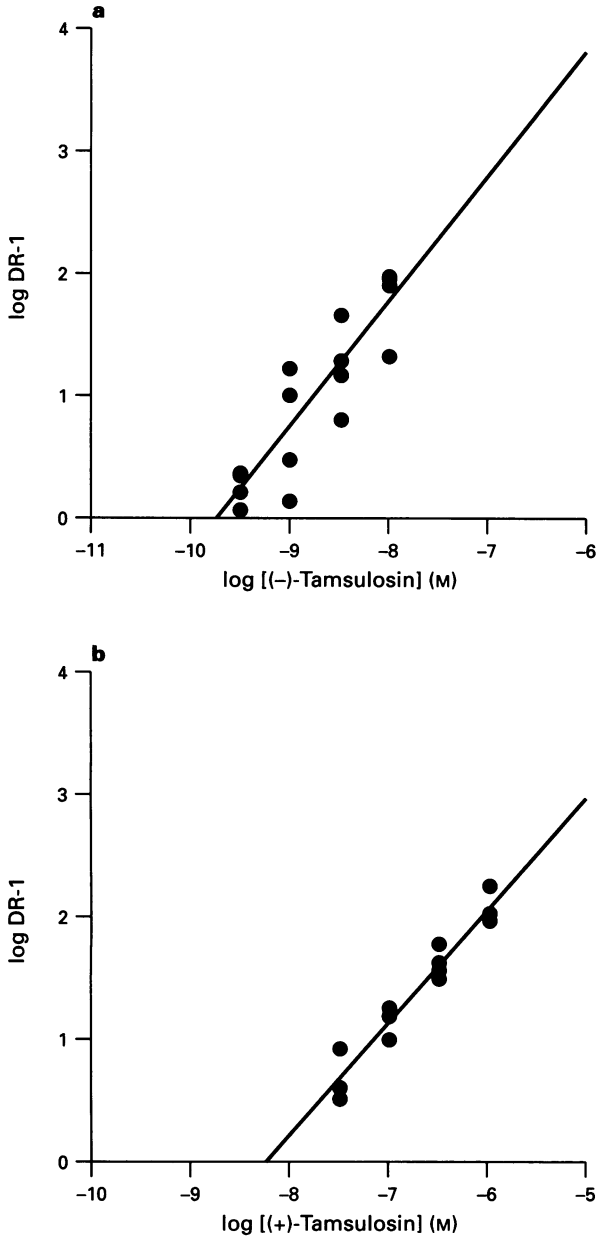

Figure 1 Schild analysis of the effects of (a) (−)-tamsulosin and (b) (+)-tamsulosin against noradrenaline-mediated contractions of rat aorta.

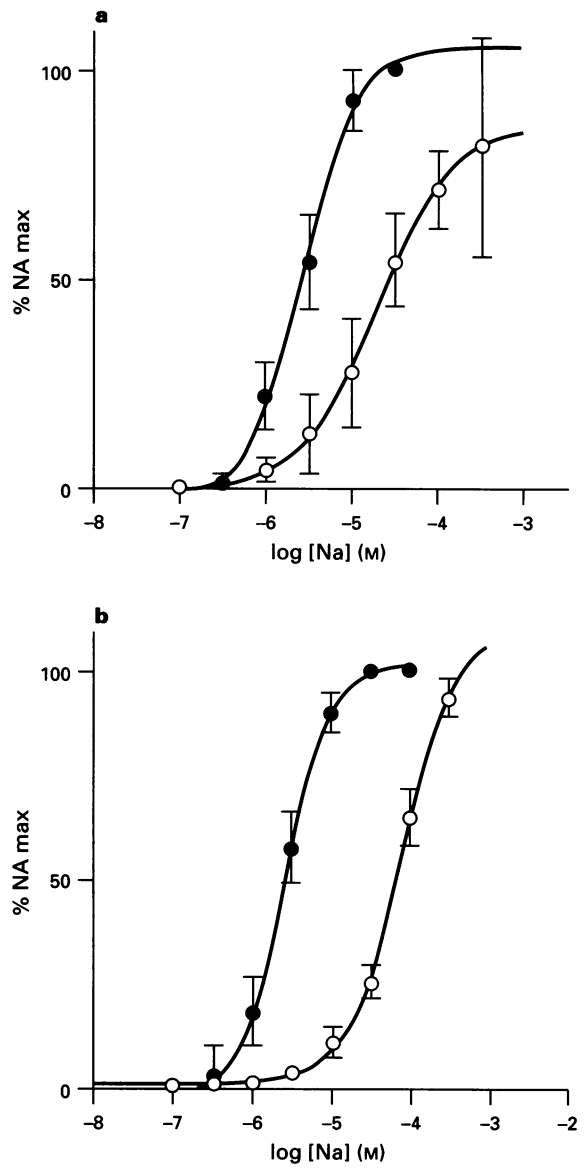

Figure 2 Comparative effects of (a) (−)-tamsulosin, 1 nM, and (b) (+)-tamsulosin, 100 nM, against noradrenaline-mediated contractions of human prostate.

Table 2 Functional affinity estimates (pA_2) for α_1 antagonists on rat aorta and human prostate

Compound	pA_2	*Rat aorta* Schild slope (95% CL)	*Human prostate* $^{\#}pA_2$ *(antagonist concentration)* μM
Prazosin*	9.8	0.93	8.7±0.27 (0.1)
Doxazosin*	8.8	1.02	8.2±0.12 (0.3)
(+)-Tamsulosin	8.23	0.93 (1.0–0.8)	8.2±0.11 (0.01)
(−)-Tamsulosin	9.70	1.02 (1.3–0.7)	9.8±0.11 (0.001)
Rec 15/2739	7.89	0.95 (0.6–1.3)	8.8±0.05 (0.003)
SL 89,0591	8.28	0.93 (0.8–1.1)	8.6±0.08 (0.1)
SNAP 1069	6.62	0.89 (0.8–1.0)	7.7±0.23 (1.0)
RS 17053	6.50	1.18 (0.8–1.5)	6.0±0.10 (3.10)

$^{\#}pA_2$ estimate determined from the inhibitory effect of a single antagonist concentration. *Data previously reported (Kenny *et al.*, 1995a) and included for comparative purposes.

nists. Phenylephrine dose-response curves were shifted to the right in parallel enabling the calculation of dose-ratios, relative to control curves, for each dose of antagonist tested (based on molar wt of compound kg^{-1} body weight). The potency of various compounds in antagonizing phenylephrine-mediated increases in prostatic pressure and blood pressure is summarized in Table 3. The non selective α_1 antagonist, doxazosin, together with (+)- and (−)-tamsulosin were all potent antagonists of phenylephrine-mediated responses, with dose-response curves on blood pressure and prostatic pressure shifted to the right in a competitive manner. Estimates of affinity (*in vivo* pseudo 'pA_2' values; Table 3) showed that these compounds inhibited both prostatic and blood pressure responses with similar affinity, implying that these agents show little or no selectivity for prostatic responses in the anaesthetized dog. SL 89,0591 and SNAP 1069 were moderately selective (3 and 7 fold respectively) for prostatic pressure relative to blood pressure. Rec 15/2739 (Figure 4) was a more potent antagonist of phenylephrine mediated increases in prostatic pressure ('pA_2' = 8.74) compared to blood pressure ('pA_2' = 7.51).

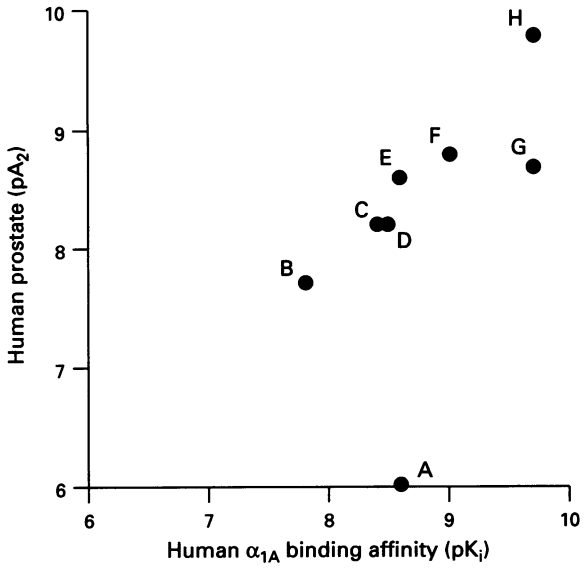

Figure 3 Comparative binding potencies for compounds (pK_i) at cloned human α_{1A} adrenoceptors and functional affinity estimates (pA_2) against noradrenaline-mediated contractions of human prostate. (A) RS 17053; (B) SNAP 1069; (C) (+)-tamsulosin; (D) doxazosin; (E) SL 89,0591; (F) Rec 15/2739; (G) prazosin; (H) tamsulosin.

Table 3 Comparative antagonist potencies for α_1 adrenoceptor antagonists as inhibitors of phenylephrine-mediated increases in prostatic pressure and blood pressure in the anaesthetized dog.

Antagonist	*Blood pressure* (pA_2, slope, 95% CL)	*Prostatic pressure* (pA_2, slope, 95% CL)
Doxazosin*	7.5	7.5
(−)-Tamsulosin	8.8 (0.95, 0.4–1.5)	8.9 (1.80, 1.3–2.2)
(+)-Tamsulosin	7.3 (0.73, 0.3–1.1)	7.4 (1.59, 0.5–2.7)
SL 89,0591	7.3 (0.86, 0.5–1.1)	7.8 (1.58, 0.9–2.2)
Rec 15/2739	7.5 (0.53, 0.3–0.8)	8.7 (0.97, 0.4–1.5)
SNAP 1069	6.7 (0.61, 0.2–1.0)	7.5 (1.45, 0.9–2.0)

Affinities were determined by Schild analysis of dose-ratios derived from shifts in the dose-response curves to phenylephrine as decribed in Methods (each dose-ratio determined from the antagonist shift meaned from 3–4 different experiments). *Value previously reported (Kenny *et al.*, 1994).

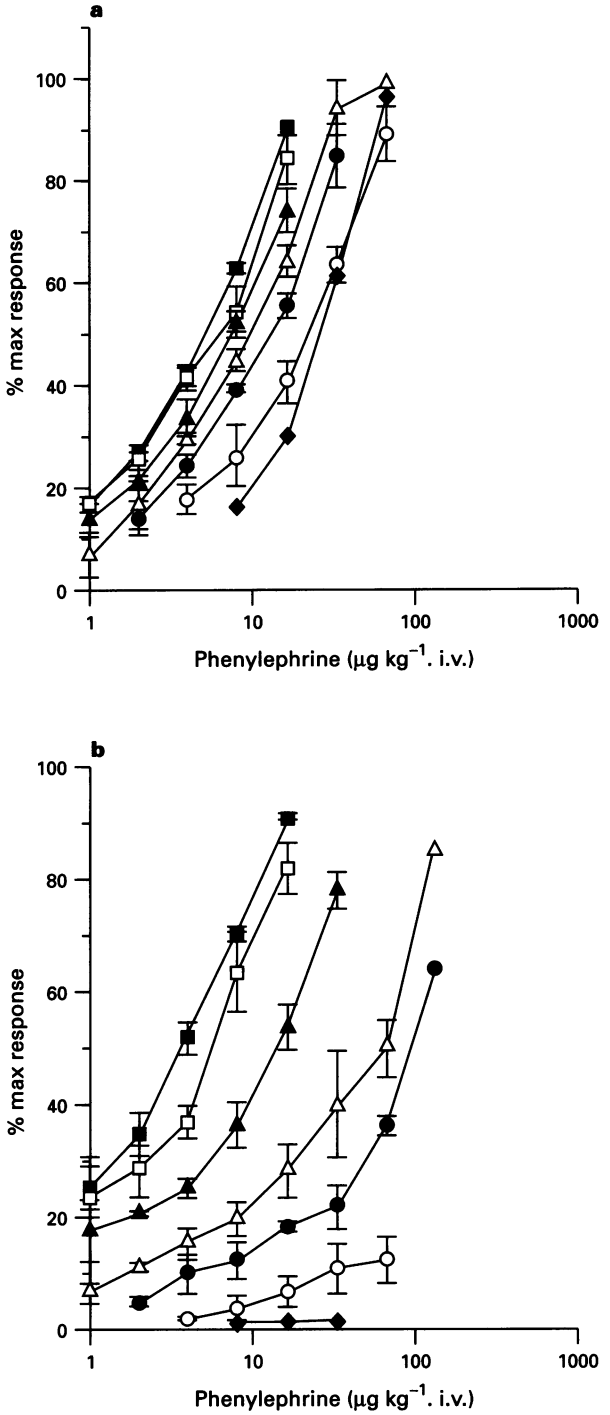

Figure 4 The effect of Rec 15/2739 on phenylephrine-induced rises in blood pressure (a) and intraurethral prostatic pressure (b) in the anaesthetized dog. Data points show the mean±s.e. mean for 3–4 separate determinations for control (■) and following Rec 15/2739 at 1 (□), 3 (▲), 10 (△), 30 (●), 100 (●) and 300 (◆) μg kg^{-1}, i.v.

Discussion

The profile of α_1 adrenoceptor antagonists obtained in the present study clearly demonstrates a predominant role for α_1 adrenoreceptors in the contractile response of both human and canine prostate and confirms previous data in which an exclusive role for α_1 rather than α_2 adrenoceptors has been described (Hieble *et al.*, 1985). However, whilst much of the data in the present study confirm previous reports suggesting a predominant role for the α_{1A} adrenoceptor in the contractile response of human prostate (Forray *et al.*, 1994; Chapple *et al.*, 1989), other findings suggest that this receptor cannot be satisfactorily designated as an α_{1A} subtype.

In several previous studies, estimates of antagonist affinity for a range of competitive α_1 antagonists has suggested that α_{1A} adrenoceptors mediate the contractile response of human prostate. A clear correlation has been demonstrated between the binding affinity of compounds for cloned human α_{1A} adrenoceptors and corresponding functional affinity estimates against α_1 mediated contractions of human prostatic smooth muscle *in vitro* (Forray *et al.*, 1994; Marshall *et al.*, 1995). Consistent with our findings, compounds with differing degrees of selectivity for the cloned α_{1A} subtype such as 5-methylurapidil, WB 4101, indoramin and SNAP 1069 exhibit antagonist affinity estimates consistent with their α_{1A} binding affinities. Furthermore, in a recent study carried out by Goetz *et al.* (1994) the profile of more than 20 compounds against [^{125}I]-HEAT in human and canine prostatic tissue homogenates was highly correlated with their affinity at cloned human α_{1A}-adrenoceptors, but not with α_{1B} or α_{1D} subtypes.

Similar conclusions have also been reached on the basis of [^{3}H]-prazosin binding to human prostatic membranes (Testa *et al.*, 1995).

However, other studies suggest that at least two α_1 adrenoceptor subtypes can be detected from binding studies to prostatic homogenates. In studies by Muramatsu *et al.*(1994) displacement studies with prazosin have been found to be biphasic, suggesting that prazosin can distinguish between two different α_1 adrenoceptor subtypes on prostatic membranes. This finding is not consistent with the profile of prazosin at currently classified α_1 adrenoceptors since this α_1 adrenoceptor antagonist does not discriminate between cloned receptor subtypes. However, in addition to prazosin, binding studies to human prostate membranes with other compounds such as oxymetazoline (Faure *et al.*, 1994) and RS 17053 (Ford *et al.*, 1995) also demonstrate multiple prostatic α_1 adrenoceptors. A key issue therefore, is how the affinity of compounds for these different sites relates to their functional antagonist affinity estimates against noradrenaline-mediated contractile responses.

Several studies suggest that the characteristics of the functional α_1 adrenoceptor on human prostate is consistent with the low affinity binding site identified by prazosin and RS 17053 (Muramatsu *et al.*, 1994; Ford *et al.*, 1995). Since the low binding and functional affinities exhibited by these compounds are not consistent with their profile at cloned α_{1A} adrenoceptors, these data suggest the existence of a distinct subtype which is functionally predominant. On this basis, the relatively low affinity exhibited by prazosin against α_1-mediated contractile responses (pA_2 affinity estimates <9.0) in contrast to the affinity for cloned α_{1A} receptors ($pK_i \geqslant 9.5$) has been suggested to be indicative of a distinct receptor, having the characteristics of the previously described α_{1L} adrenoceptor (Muramatsu *et al.*, 1990). The profile of the potent and selective α_{1A} antagonist, RS 17053, obtained in our study confirms the previous report by Ford *et al.* (1995) and clearly suggests that the cloned α_{1A} adrenoceptor and the α_1 subtype mediating contractile responses of human prostate *in vitro* are different. If the prostatic α_1 adrenoceptor mediating contractile responses to noradrenaline is not entirely consistent with its designation as a typical α_{1A} adrenoceptor, it appears to be at least closely related. Thus far, exhaustive attempts have failed to identify additional α_1 adrenoceptor subtypes using homology based molecular cloning approaches. However, genes encoding the α_1 adrenoceptor possess introns and, recently, splice variants of the α_{1A} adrenoceptor have been described (Hirasawa *et al.*, 1995). Whether such a splice variant accounts for the pharmacological profile exhibited by human prostate in contractile studies remains to be determined.

Receptor binding affinities for a range of compounds at cloned α_{1D} adrenoceptors in comparison to antagonist affinity estimates on rat aorta suggest a predominant role for α_{1D} adrenoceptors in the contractile response to noradrenaline, despite strong evidence for heterogeneity of the α_1 population in this tissue (Kenny *et al.*, 1995a). In this current study, affinity estimates on rat aorta for compounds also compare well with α_{1D} binding affinities, confirming previous observations. Thus, we have used a combination of binding and functional *in vitro* data to determine the selectivity of compounds for human prostatic α_1 adrenoceptors in comparison to α_{1A}, α_{1B} and α_{1D} subtypes. We found that several α_1 adrenoceptor antagonists displayed selectivity for the α_{1A} subtype consistent with previous reports for RS 17053 (Ford *et al.*, 1995), Rec 15/2739 (Testa *et al.*, 1995), SNAP 1069 and indoramin (Forray *et al.*, 1994) and tamsulosin (Michel & Insel, 1994) although with the exception of RS 17053 none of these particular compounds show selectivity for α_{1A} adrenoceptors over both α_{1B} and α_{1D} subtypes. Thus, Rec 15/2739, (+)- and (−)-tamsulosin were selective for α_{1A} adrenoceptors relative to the α_{1B} subtype. In contrast we found that indoramin and SNAP 1069 showed selectivity for α_{1A} adrenoceptors relative to the α_{1D} subtype. More importantly, most of these agents also displayed relative selectivity for human prostate *in vitro* on a similar basis to their selectivity for the α_{1A} subtype although the insurmountable behaviour of (−)-tamsulosin *in vitro*, which has also been reported on other tissues such as rat and human vas deferens (Furukawa *et al.*, 1995), precluded an accurate affinity estimate for this compound on human prostate. Given the differing profiles of these α_1 adrenoceptor antagonists, we have therefore attempted to elucidate the importance of their *in vitro* prostatic selectivity using an anaesthetized dog model allowing the simultaneous assessment of blood pressure and prostatic pressure (Kenny *et al.*, 1994). This study therefore extends previous reports showing that α_1 adrenoceptor antagonists decrease urethral resistance in animal models *in vivo* (Breslin *et al.*, 1993; Lefevre-Borg *et al.*, 1993) consistent with clinical reports conferring improvements in urinary flow with these agents (Wilde *et al.*, 1993).

Changes in intraurethral pressure, measured in the prostatic portion, enable the measurement of changes in urethral pressure as a direct reflection of prostatic tone rather than changes in urethral tone *per se*. Other studies in a highly comparable model also conclude that changes in intraurethral pressure measured in the prostatic portion are primarily due to contraction of the prostate gland rather than urethral smooth muscle (Brune *et al.*, 1995), although contractions of urethral tissue are also mediated by α_1 adrenoceptors. An *in vivo* derived pA_2 does not quantitatively estimate an antagonist affinity constant since all the criteria needed for the application of Schild analysis are not strictly adhered to. Thus, Schild regression deviating from unity may reflect lack of dynamic equilibrium between drug and tissue/receptor following *in vivo* administration. In addition, rises in blood pressure following agonist administration (and its subsequent antagonism) as a reflection of increased peripheral resistance cannot be accounted for on the basis of single receptor kinetics since multiple subtypes are likely to contribute to this response, probably to different extents. Nevertheless, this model does provide a relevant measurement of potency and comparative tissue selectivity for compounds against simultaneous agonist challenge on two parameters, although it must be noted that there is considerable species variation in the distribution and function of vascular α_1 adrenoceptor subtypes. Therefore, blood pressure increases as a function of vascular resistance should be considered an integrated response. Using this model we have previously shown that compounds without any intrinsic selectivity between α_1 adrenoceptor subtypes were equipotent against blood pressure and prostatic pressure responses (Kenny *et al.*, 1994). The profile of several α_1 adrenoceptor antagonists on prostatic pressure in the anaesthetized dog has recently been reported (Brune *et al.*, 1995), and of those agents common to our studies, antagonist profiles are highly comparable.

In the context of selectivity, conclusions based on the profile of several compounds in our model are not entirely consistent with some other studies, particularly with tamsulosin. Clearly, this compound is selective for the α_{1A} subtype relative to the α_{1B} adrenoceptor, although against cloned human α_1 adrenoceptors, we found that neither enantiomer showed selectivity over the α_{1D} subtype. Similar conclusions can be drawn on the basis of affinity estimates in rat aorta and human prostate. In our canine model this antagonist shows a balanced profile against prostatic pressure and blood pressure *in vivo*, with the (−)-isomer considerably more potent than the corresponding (+)-enantiomer. These data are similar to the finding of Shibaski *et al.* (1992) who showed that (−)-tamsulosin was several orders of magnitude more potent than the corresponding (+)-enantiomer. They also found that both enantiomers displayed similar degrees of selectivity, similar to prazosin, against agonist-induced increases in urethral pressure and blood pressure, although those studies were carried out in the anaesthetized female dog (and therefore assess increases in urethral pressure *per se*). The profile of several α_1 adrenoceptor antagonists in the anaesthetized male dog has recently been described in which greater prostatic selectivity than we observed was reported for both tamsulosin and Rec 15/2739 (10 fold and 100 fold respectively; Testa *et al.*, 1994). There are a

number of reasons for these differences compared to our data. Firstly, we have used phenylephrine as an agonist rather than noradrenaline to reduce or eliminate any contribution from non α_1 adrenoceptors. In addition, we have determined antagonist potency on the basis of shifts in phenylephrine-response curves for both blood pressure and prostatic pressure, allowing a direct comparison between antagonist potency on these two parameters (in contrast to antagonist effects on blood pressure *per se*). We therefore found much less variability between blood pressure measurements under this protocol than in the study reported by Testa *et al.* (1994). Nevertheless, both this and our own study have shown that Rec 15/2739 (and SL 89,0591 to a lesser extent) are inherently more selective than agents such as doxazosin and tamsulosin.

Taken together, our data indicate that selectivity for prostatic α_1 adrenoceptors relative to the α_{1B} subtype is not sufficient to confer selectivity *in vivo* since both tamsulosin and Rec 15/2739 show this profile, with *in vivo* selectivity only apparent with the latter compound. On the basis of the data in this study, selectivity for the prostatic α_1 adrenoceptor relative to the α_{1D} subtype appears a better index of prostatic/vascular selectivity, at least in this model. In the context of BPH, compounds with optimized potency at the prostatic α_1 adrenoceptor with minimal vascular actions are clearly desirable and data obtained in our study suggest that whilst prostatic potency can be easily quantified in isolation, selectivity *in vivo* needs to be carefully assessed since multiple subtypes are likely to contribute to vascular responses.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–52.

BRESLIN, D., FIELDS, D.W., CHOU, T-C., MARION, D.N., KANE, M., VAUGHAN, E.D. & FELSON, D. (1993). Medical management of benign prostatic hyperplasia: A canine model comparing the *in vivo* efficacy of alpha-1 adrenergic antagonists in the prostate. *J. Urol.*, **149**, 395–399.

BRUNE, M.E., BUCKNER, S.A., POLAKOWSKI, J., KERWIN, J.F. & HANCOCK, A. (1995). Pharmacological antagonism of α adrenergic agonist induced increases in canine intraurethral pressure *in vivo*. *Drug Dev. Res.*, **34**, 267–275.

CHAPPLE, C.R., AUBREY, M.L. & JAMES, S. (1989). Characterization of human prostatic adrenoceptors using pharmacology receptor binding and localisation. *Br. J. Urol.*, **63**, 487–496.

FAURE, C., PIMOULE, C., VALLANCIEN, G., LANGER, S.Z. & GRAHAM, D. (1994). Identification of α_1 adrenoceptor subtypes present in the human prostate. *Life Sci.*, **54**, 1595–1605.

FLAVAHAN, N.A. & VANHOUTTE, P.M. (1986). α_1 Adrenoceptor subclassification in vascular smooth muscle. *Trends Pharmacol. Sci.*, **7**, 347–349.

FORD, A.P.D.W., ARREDONDO, N.F., BLUE, D.R. Jr., BONHAUS, D.W., KAVA, M.S., WILLIAMS, T.J., VIMONT, R.L., ZHU, Q.M., PFISTER, J.R. & CLARKE, D.E. (1995). Do alpha-1A (alpha-1C)-adrenoceptors (AR) mediate prostatic smooth muscle contraction in man? Studies with a novel, selective alpha-1A-AR antagonist, RS 17053. *Br. J. Pharmacol.*, **114**, 24P.

FORD, A.P.D.W., WILLIAMS, T.J., BLUE, D.R. & CLARKE, D.E. (1994). α_1 Adrenoceptor classification: sharpening Occam's razor. *Trends Pharmacol. Sci.*, **15**, 167–170.

FORRAY, C., BARD, J.A., WETZEL, J.M., CHIU, G., SHAPIRO, E., TANG, R., LEPOR, H., HARTIG, P.R., WEINSHANK, R.L., BRANCHEK, T.A. & GLUCHOWSKI, C. (1994). The α_1 adrenoceptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1c} subtype. *Mol. Pharmacol.*, **45**, 703–708.

FURUKAWA, K., CHESS-WILLIAMS, R., NOBLE, A.J., ROSARIO, D.J., CHAPPLE, C.R. & UCHIYAMA, T. (1995). Non-surmountable antagonist effects of tamsulosin on the α_{1A} adrenoceptor-mediated responses of the rat and human vas deferens. *Br. J. Pharmacol.*, **115**, 127P.

GOETZ, A.S., LUTZ, M.W., RIMELE, T.J. & SAUSSY, D.L. (1994). Characterization of alpha-1 adrenoceptor subtypes in human and canine prostate membranes. *J. Pharmacol. Exp. Ther.*, **271**, 1228–1233.

HIEBLE, J.P., BYLUND, D.B., CLARKE, D.E., EIKENBURG, D.C., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P. & RUFFOLO, R.R. (1995). Recommendation for nomenclature of α_1 adrenoceptors: consensus update. *Pharmacol Rev.*, **47**, 267–270.

HIEBLE, J.P., CAINE, M. & ZALAZNIK, E. (1985). *In vitro* characterization of the alpha-adrenoceptors in human prostate. *Eur. J. Pharmacol.*, **107**, 111–117.

HIRASAWA, A., SHIBATA, K., HORIE, K., TAKEI, Y., OBIKA, K., TANAKA, T., MURAMOTO, N., TAKAGAKI, K., YANO, J. & TSUJIMOTO, G. (1995). Cloning, functional expression and tissue distribution of human α_{1C}-adrenoceptor splice variants. *FEBS Lett.*, **363**, 256–260.

KENNY, B.A., CHALMERS, D.H., PHILPOTT, P.C. & NAYLOR, A.M. (1995a). Characterization of an α_{1D} adrenoceptor mediating the contractile response of rat aorta to noradrenaline. *Br. J. Pharmacol.*, **115**, 981–986.

KENNY, B.A., COLLIS, A., NAYLOR, A. & WYLLIE, M. (1995b). α_1-Adrenoceptor antagonists as treatments for benign prostatic hyperplasia. *Exp. Opinion Invest. Drugs*, **4**, 915–923.

KENNY, B.A., NAYLOR, A.M., CARTER, A.J., READ, A.M., GREENGRASS, P.M. & WYLLIE, M.G. (1994). Effect of alpha-1 adrenoceptor antagonists on prostatic pressure and blood pressure in the anaesthetised dog. *Urology*, **44**, 52–57.

KOBAYASHI, S., TANG, R., SHAPIRO, E. & LEPOR, H. (1993). Characterisation and localization of prostatic alpha$_1$ adrenoceptors using radioligand binding on slide-mounted tissue sections. *J. Urol.*, **150**, 2002–2006.

LEFEVRE-BORG, F., O. CONNOR, S.E., SCHOEMAKER, H., HICKS, P.E., LECHAIRE, J., GAUTIER, E., PEEIRRE, F., PIMOULE, C., MANOURY, P. & LANGER, S.Z. (1993). Alfuzosin, a selective α_1 adrenoceptor antagonist in the lower urinary tract. *Br. J. Pharmacol.*, **109**, 1282–1289.

LEPOR, H., TANG, R. & SHAPIRO, E. (1993). The alpha-adrenoceptor mediating the tension of human prostatic smooth muscle. *Prostate*, **22**, 301–307.

MARSHALL, I., BURT, R.P. & CHAPPLE, C.R. (1995). Noradrenaline contractions of human prostate mediated by α_{1A}-(α_{1c}-)adrenoceptor subtype. *Br. J. Pharmacol.*, **115**, 781–786.

MCNEAL, J. (1990). Pathology of benign prostatic hyperplasia: insight into etiology. *Urol. Clin. North Am.*, **17**, 477–486.

MICHEL, M.C. & INSEL, P.A. (1994). Comparison of cloned and pharmacologically defined rat tissue α_1-adrenoceptor subtypes. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **350**, 136–142.

MICHEL, M.C., KENNY, B.A. & SCHWINN, D.A. (1995). Classification of α_1 adrenoceptor subtypes. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **352**, 1–10.

MURAMATSU, I., OHMURA, T., KIGOSHI, S., HASHIMOTO, S. & OSHITA, M. (1990). Pharmacological subclassification of α_1 adrenoceptors in vascular smooth muscle. *Br. J. Pharmacol.*, **99**, 197–201.

MURAMATSU, I., OSHITA, M., OHMURA, T., KIGOSHI, S., AKINO, H. & OKADA, K. (1994). Pharmacological characterisation of α_1 adrenoceptor subtypes in the human prostate; functional and binding studies. *Br. J. Urol.*, **74**, 572–578.

PRICE, D.T., SCHWINN, D.A., LOMASNEY, J.W., ALLEN, L.F., CARON, M.G. & LEFKOWITZ, R.J. (1993). Identification, quantitation, and localization of mRNA for three distinct alpha$_1$ adrenergic subtypes in human prostate. *J. Urol.*, **150**, 546–551.

RUFFOLO, R.R., NICHOLS, A.J., STADEL, J.M. & HIEBLE, J.P. (1991). Structure and function of α-adrenoceptors. *Pharmacol. Rev.*, **43**, 475–506.

SCHWINN, D.A., JOHNSTON, G.I., PAGE, S.O., MOSLEY, M.J., WILSON, K.H., WORMAN, N.P., CAMPBELL, S., FIDOCK, M.D., FURNESS, L.M., PARRY-SMITH, D.J., PETER, B. & BAILEY, D.S. (1995). Cloning and pharmacological characterisation of human α_1 adrenergic receptors: sequence corrections and direct comparison with other species homologs. *J. Pharmacol. Exp. Ther.*, **272**, 134–142.

SHIBASKI, M., SUDOH, K., INAGAKI, O., UCHIDA, W. & HONDA, K. (1992). Effect of optical isomers of YM-12617 on increased intraurethral pressure induced by phenylephrine in anaesthetised dogs. *J. Autonom. Pharmacol.*, **12,** 263–268.

TESTA, R., SIRONI, G., COLOMBO, D., GRETO, L. & LEONARDI, A. (1994). Rec 15/2739, a new α_1 adrenoceptor antagonist selective for the lower urinary tract: *in vivo* studies. *Neurourol. Urodynamics*, **13,** 471–472.

TESTA, R., TADDEI, C., POGGESI, E., DESTEFANI, C., COTECCHIA, S., HIEBLE, J.P., SULPIZIO, A.C., NASELSKY, D., BERGSMA, D., ELLIS, C., SWIFT, A., GANGULY, S., RUFFOLO, R.R. & LEONARDI, A. (1995). Rec 15/2739 (SB 216469): A novel prostate selective α_1 adrenoceptor antagonist. *Pharmacol. Commun.*, **6,** 79–86.

WILDE, M.I., FITTON, A. & McTAVISH, D. (1993). Alfuzosin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in benign prostatic hyperplasia. *Drugs*, **45,** 410–429.

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