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The α-adrenoceptor antagonist properties of the enantiomers of doxazosin in the human prostate

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Abstract

The α-adrenoceptor antagonist properties of doxazosin and its enantiomers were characterized using human prostate tissue and cell membranes isolated from rat-1 fibroblast expressing each of the cloned human α_1-adrenoceptor subtypes. In the α_1-adrenoceptor-binding studies on the human prostate with [^{3}H]doxazosin and 2-{[β-(3-[^{125}I],4-hydroxyphenyl)ethyl]aminomethyl}-1-tetralone ([^{125}I]HEAT), no significant differences were observed between racemic doxazosin, *R*-doxazosin and *S*-doxazosin (mean $-\log K_i$ (pK_i) values were 8.60–8.63, 8.47–8.55 and 8.61–8.65, respectively), whereas the α_2-adrenoceptor-binding studies with [^{3}H]rauwolscine and [^{3}H]clonidine revealed that the α_2-adrenoceptor-binding affinity of *S*-doxazosin ($pK_i = 5.91–5.94$) was slightly (3- or 4-fold), but significantly lower than that of *R*-doxazosin ($pK_i = 6.47–6.54$). Studies in phenylephrine-contracted prostatic tissue showed no significant difference in α_1-adrenoceptor antagonist potency between racemic doxazosin, *R*-doxazosin and *S*-doxazosin (pA_2 values were 8.43 ± 0.28, 8.64 ± 0.56 and 8.75 ± 0.38, respectively). In the binding studies with cloned α_1-adrenoceptor subtypes using [^{3}H]prazosin and [^{125}I]HEAT, racemic doxazosin, *R*-doxazosin and *S*-doxazosin showed no selectivity for the α_1-adrenoceptor subtypes. The present study demonstrated that doxazosin and its enantiomers are highly selective α_1-adrenoceptor antagonists and that there is no evidence suggesting differential α_1-adrenoceptor antagonist effects of doxazosin and its enantiomers in the human prostate. Doxazosin, therefore, could be described as displaying balanced activity across all three α_1-adrenoceptor subtypes.

Keywords: α-Adrenoceptor; Prostate; cDNA; Fibroblast, rat-1; Doxazosin; (Enantiomer)

1. Introduction

Medical therapy of symptomatic benign prostatic hyperplasia based on the pharmacological blockade of prostatic α-adrenoceptor was introduced nearly two decades ago (Caine et al., 1976). The rationale for the α-adrenoceptor blockade originated from an analysis of the pharmacology of prostatic contraction in vitro which showed that α-adrenoceptors mediate contraction of the prostate (Caine et al., 1975). Although radioligand-binding experiments revealed that the densities of α_1- and α_2-adrenoceptors are similar in human prostate adenomas (Lepor and Shapiro, 1984; Shapiro and Lepor, 1986; Gup et al., 1990), isolated organ studies demonstrated that it was primarily the α_1-adrenoceptors which mediated contraction of the human prostate (Lepor and Shapiro, 1984; Hieble et al., 1985; Shapiro and Lepor, 1986; Lepor et al., 1988a). Antagonists, such as phentolamine and phenoxybenzamine, which do not discriminate among α_1- and α_2-adrenoceptors, antagonize pre-synaptic autoreceptors increasing circulatory and synaptic levels of noradrenaline resulting in tachycardia, hyperreninemia and attenuation of the therapeutically desired post-synaptic blockade (Graham and Pettinger, 1979; Langer et al., 1980; Saeed et al., 1982). Thus, medical therapy for benign prostatic hyperplasia has been directed toward selective α_1-adrenoceptor antagonism. While selective α_1-adrenoceptor antagonists, such as prazosin, alleviate the urinary symptoms associated with benign prostatic hyperplasia, the development of systemic side effects in some subjects limits dose escalation and the maximum therapeutic effect may not be achieved. The most common adverse events associated with α_1-adrenoceptor antago-

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nists are hypotension, dizziness, fatigue and light-headedness (Lepor et al., 1990a; Fulton et al., 1995), which may arise from non-specific antagonism of the vascular and cerebral α_1-adrenoceptors.

Recent pharmacological and molecular biological research has demonstrated heterogeneity of α_1-adrenoceptors. At least three native α_1-adrenoceptors have been identified pharmacologically, and three distinct α_1-adrenoceptors have been cloned (Bylund et al., 1994). The relationship between the native and recombinant α_1-adrenoceptor subtypes has only recently been elucidated (Ford et al., 1994). Thus, current α_1-adrenoceptor subclassification recognizes three subtypes which have been designated α_{1A}, α_{1B} and α_{1D}, with the corresponding cloned subtypes designated with lower-case letters α_{1a}, α_{1b} and α_{1d} (Hieble et al., 1995). Hirasawa et al. (1993) first cloned the human α_{1a}-adrenoceptor cDNA from human prostate. All three α_1-adrenoceptor mRNAs have been shown to be expressed in the human prostate, but the α_{1a}-mRNA has been shown to be predominant in the prostatic stroma (Price et al., 1993; Tseng-Crank et al., 1995). Reports from several laboratories have shown that the ability of a series of selective antagonists to inhibit the contraction of human prostate smooth muscle correlates highly with their affinity for inhibition of radioligand binding to the recombinant α_{1a}-adrenoceptor subtype (Forray et al., 1994; Marshall et al., 1995). These results strongly suggest that the contractile response of the human prostate is mediated mainly by an α_{1A}-adrenoceptor. Also in the vasculature, all three subtypes are found to varying extents. However, several studies on α_1-adrenoceptor subtypes in human tissues suggest that the α_1-adrenoceptor subtype which predominantly mediates the contraction of human aorta and peripheral artery may be different from that in the human prostate, by use of RNase protection (Price et al., 1994), radioligand receptor binding (Yamada et al., 1994) and isolated organ bath techniques (Hatano et al., 1994). On this basis, selective targeting of the subtype primarily involved in prostatic contraction (α_{1A}) should reduce the degree of cardiovascular changes and offer a theoretical clinical advantage. To explore further the opportunity to achieve subtype selectivity, the stereochemical specificity of the enantiomers of α_1-adrenoceptor antagonists for α-adrenoceptor-binding sites has been investigated. As α_1-adrenoceptor antagonists used for the treatment of benign prostatic hyperplasia, the two optical isomers of YM-12617 (tamsulosin) (Honda and Nakagawa, 1986; Honda et al., 1987; Lepor et al., 1988b) and terazosin (Kyncl et al., 1990; Meretyk et al., 1992) have already been reported to have differential α_1/α_2 adrenoceptor selectivity. Furthermore, *R*-(−)-YM-12617, the more potent enantiomer of YM-12617, displays selectivity for the α_1-adrenoceptor subtypes (Knepper et al., 1995).

Doxazosin is a quinazoline derivative structurally related to prazosin and terazosin (Fig. 1), and a long-acting α_1-adrenoceptor antagonist (Fulton et al., 1995). Several

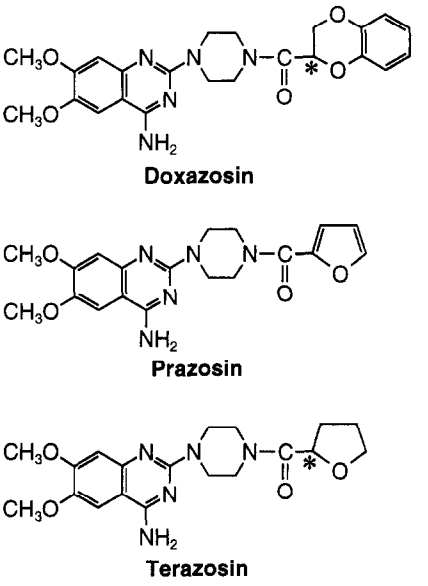

Fig. 1. Structural formulas of the α_1-adrenoceptor antagonists doxazosin, prazosin and terazosin. Asterisk denotes the point of asymmetry. Prazosin has no chiral center. Doxazosin and terazosin have one asymmetric carbon in the 1,4-benzodioxan moiety and tetrahydrofuran moiety, respectively, therefore two optical enantiomers exist for each compound.

controlled clinical studies have demonstrated that doxazosin is an effective, safe and well-tolerated drug for the treatment of symptomatic benign prostatic hyperplasia (Fulton et al., 1995). Doxazosin has an asymmetric carbon at the 2 position of the 1,4-benzodioxan-2-ylcarbonyl ring, and two optical isomers, i.e. *R*- and *S*-forms, exist. Despite the clinical efficacy of doxazosin, the α-adrenoceptor antagonist properties of these enantiomers have not previously been characterized in detail. The objective of the present study was to characterize the α-adrenoceptor antagonist properties of doxazosin and its enantiomers in the human prostate and cloned human α_1-adrenoceptor subtypes. Differential pharmacological properties of the enantiomers of doxazosin may have implications in the design of a new generation of prostate-selective α_1-adrenoceptor antagonists for the treatment of benign prostatic hyperplasia. This study extends the work of Lepor et al. (1990b).

2. Materials and methods

2.1. Chemicals

[^{3}H]Prazosin (specific activity 2.7528 TBq/mmol), [^{125}I]HEAT (2-{[β-(3-[^{125}I],4-hydroxyphenyl)ethyl]aminomethyl}-1-tetralone) (specific activity 81.4 TBq/mmol), [^{3}H]rauwolscine (specific activity 2.9785 TBq/mmol) and [^{3}H]clonidine hydrochloride (specific activity 2.5456 TBq/mmol) were obtained from Dupont/New England Nuclear (Boston, MA, USA). Doxazosin mesylate (racemate), *R*-doxazosin (UK-36,528-27), *S*-doxazosin (UK-35,494-27) and [^{3}H]doxazosin methane sulphonate

(specific activity 1.04 TBq/mmol) were donated by Pfizer Central Research (Sandwich, Kent, UK). Terazosin hydrochloride was obtained from Abbott Laboratories (Abbott Park, IL, USA). Phenylephrine hydrochloride, 5-methylurapidil and phentolamine mesylate were obtained from Research Biochemicals International (Natick, MA, USA).

2.2. Radioligand-binding studies on human prostate

2.2.1. Tissue specimens

Radioligand receptor-binding assays using human prostate were performed on slide-mounted tissue section according to the method of Kobayashi et al. (1993). Human prostatic tissue was obtained from the benign elements of the transition zone of eight patients with low volume prostate cancer undergoing radical prostatectomies. The tissue specimens were immediately transferred into a −80°C freezer for storage. The frozen tissue sections were cut into an approximately rectangular configuration such that the weight of a single tissue section was estimated by the following equation: (length) × (width) × (thickness) × (tissue density). The prostatic tissues were embedded in O.C.T. compound and 20-μm-thick tissue sections were cut using a cryostat set at −20°C. The slide-mounted tissue sections were stored at −80°C until the binding assays were performed.

2.2.2. Optimal assay conditions; pre-incubation time

Preincubation may be necessary to maximize specific to non-specific binding of ligand and to remove endogenous catecholamines from the samples. The requirement for pre-incubation is ligand dependent and needs to be determined empirically for each ligand (Kuhar and Unnerstall, 1990). In the present study, [^{3}H]prazosin- and [^{125}I]-HEAT-binding assays were carried out to characterize α_1-adrenoceptor-binding properties of unlabeled doxazosin racemate (*rac*-doxazosin), its enantiomers and terazosin racemate (*rac*-terazosin). [^{3}H]Rauwolscine and [^{3}H]clonidine were used to characterize α_2-adrenoceptor-binding properties of these competitors. To determine the requirement for pre-incubation in the binding experiments on the slide-mounted human prostatic tissue sections using [^{3}H]doxazosin, [^{125}I]HEAT, [^{3}H]rauwolscine and [^{3}H]-clonidine, total and non-specific binding of each radioligand was determined at varying pre-incubation intervals. The assays were performed in triplicate. After storage, the tissue sections were brought to room temperature and pre-incubated in Tris buffer (50 mM Tris · HCl, 10 mM $MgCl_2$, pH 7.4) for 0, 5, 10, 20, 40 or 60 min. The tissue sections were incubated for 60 min at room temperature in 100 μl of the appropriate ligand solution. Ligand solutions comprised fixed concentrations of [^{3}H]doxazosin (2.8 and 20 nM), [^{125}I]HEAT (0.04 and 0.4 nM), [^{3}H]rauwolscine (0.5 and 4.0 nM) or [^{3}H]clonidine (6.0 and 40 nM). Total binding was determined by immersing the prostatic tissue sections in constant concentrations of the radioligands, and non-specific binding was determined in parallel experiments using the ligand solution with 10 μM phentolamine. Immediately after incubation, the tissue sections were briefly rinsed and then washed once in ice-cold Tris buffer for 5 min. The tissue sections were removed from the slides using Q-tip swabs. Tritium was determined by liquid scintillation counting after immersing the Q-tips in scintillation cocktail overnight. In the experiments using [^{125}I]HEAT, the dpm values of the Q-tips were counted with an automatic γ-counter.

2.2.3. Incubation time

The optimal incubation interval was determined by measuring total and non-specific binding of the radioligand at various incubation intervals between 1 and 60 min. The studies were performed at two constant concentrations of each radioligand as described above.

2.2.4. Washing time

The optimal washing time was determined by measuring total and non-specific binding of the radioligand at various washing intervals. The washing procedure consisted of a brief rinse followed by washing for various intervals (1–20 min) in the ice-cold Tris buffer. The studies were performed at two constant concentrations of each radioligand as described above.

2.2.5. Competitive binding studies

Competitive binding experiments were performed on the slide-mounted tissue sections in the presence of a constant final concentration of a radioligand (6 nM [^{3}H]doxazosin, 0.1 nM [^{125}I]HEAT, 2 nM [^{3}H]rauwolscine or 16 nM [^{3}H]clonidine) and 12 different concentrations of an unlabeled competitor, *rac*-doxazosin, *R*-doxazosin, *S*-doxazosin or *rac*-terazosin (0.01 nM to 3.0 μM for [^{3}H]doxazosin and [^{125}I]HEAT binding; 3.0 nM to 1.0 mM for [^{3}H]rauwolscine and [^{3}H]clonidine binding). The assays were performed in triplicate for each concentration of competitors. Following a 20-min pre-incubation in Tris buffer ([^{3}H]doxazosin and [^{125}I]HEAT experiments only), the slide-mounted tissue sections were incubated in 100 μl of ligand solution for 30 min ([^{125}I]HEAT binding) or 40 min ([^{3}H]doxazosin, [^{3}H]rauwolscine and [^{3}H]clonidine) at room temperature. Non-specific determinations were performed in parallel assays which contained 10 μM phentolamine. Immediately following the incubation, the tissue sections were briefly rinsed and washed once in the ice-cold Tris buffer for 2 min ([^{3}H]rauwolscine and [^{3}H]clonidine) or 4 min ([^{3}H]doxazosin) or 10 min ([^{125}I]HEAT). The tissue sections were removed from the slides with Q-tip swabs and the dpm values were measured as described above. Specific binding was determined by subtracting the non-specific binding component from total radioligand binding at the various competitor concentrations. Data were analyzed by a computerized non-linear regression program (PRISM; GraphPad Software, San Diego, CA, USA).

2.3. Isometric tension studies

Human prostatic tissue was obtained from the benign elements of the transition zone of nine patients with low volume prostate cancer undergoing radical prostatectomies. These tissues were placed in ice-cold Krebs solution (130 mM NaCl, 15 mM $NaHCO_3$, 5 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11.4 mM dextrose) before experimentation. The tissues were cut into strips approximately 15 mm in length and 200 mg in weight. These tissue strips were suspended in 5-ml organ baths containing Krebs buffer at 2 *g* resting tension (as determined by prior length tension studies). The baths were continuously bubbled with 95% O_2/5% CO_2 and thermoregulated to 37°C. Isometric tension was measured with Grass FT03C force displacement transducers and recorded with a computer-based oscillograph and data acquisition system (CODAS; DATAQ Instruments, Akron, OH, USA).

After a 1-h resting tension equilibration, the tissues were challenged with 150 mM KCl. Prostatic tissue strips were then exposed to cumulative concentrations of phenylephrine (1×10^{-8} to 1×10^{-2} M). Phenylephrine concentration-response experiments were performed in the absence or presence of an antagonist, doxazosin or each of its enantiomers (1×10^{-8} to 1×10^{-6} M). Before the addition of phenylephrine, the tissue was equilibrated with antagonist for 30 min. The competitive antagonistic activities were expressed as pA_2 values which were calculated from the Schild plots (Arunlakshana and Schild, 1959).

2.4. Radioligand-binding studies on the cloned human α_1-adrenoceptor subtypes

2.4.1. Source of cell lines and cell culture

Stably transfected rat-1 fibroblast cell lines expressing each of the human α_1-adrenoceptor subtypes were provided by Dr. Paul Hayter, Pfizer Central Research (Sandwich, Kent, UK). These cell lines had been transfected with the expression vector pcDNA3 containing the full-length cDNA constructs encoding each of the human α_1-adrenoceptor subtypes. The cells were grown as monolayers in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY, USA) containing 25 mM glucose and supplemented with 5% fetal bovine serum, penicillin G (5 U/ml), streptomycin sulfate (5 μg/ml) and gentamycin [G418 (350 μg/ml)] in a 10% CO_2 atmosphere.

2.4.2. Membrane preparation

Transfected cells in culture dishes were washed with phosphate buffer saline, scraped into 5 ml of 5 mM Tris · HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C to remove unbroken cells, and the particulate supernatant was centrifuged at $30000 \times g$ (15000 rpm in a Sorvall RC-5 centrifuge using a Sorvall SA-600 rotor) for 30 min at 4°C. The pellet was homogenized in 50 mM Tris · HCl, 1 mM $MgCl_2$, 0.1% ascorbic acid, pH 7.5, and after measurement of protein concentration, stored at −80°C until the binding assays were performed. Protein concentration was determined by a colorimetric assay using a commercial kit, with bovine plasma γ-globulin as the standard (Bio-Rad Laboratories, Hercules, CA, USA).

2.4.3. Competitive binding studies

Two separate radioligands, [^{3}H]prazosin and [^{125}I]-HEAT, were used for the receptor-binding assays using membrane preparations from the transfected rat-1 fibroblasts. Competitive binding experiments were performed in the presence of a constant final concentration of a radioligand (0.5 nM [^{3}H]prazosin or 0.08 nM [^{125}I]HEAT) and 18 different, increasing concentrations of an unlabeled competitor, *rac*-doxazosin, *R*-doxazosin, *S*-doxazosin or 5-methylurapidil (0.3 pM to 100 μM). Binding was performed in a final volume of 250 μl in glass (for [^{3}H]prazosin binding) or polypropylene (for [^{125}I]HEAT binding) test tubes. The assays were repeated in triplicate for each concentration of competitors. Membrane preparations were incubated on a shaker for 60 min at 25°C. Non-specific binding was determined in the presence of 10 μM phentolamine. The concentration of membrane protein was adjusted for each receptor subtype so that the total bound radioligand did not exceed 10% of the radioactivity added to the reaction mixture. The binding assays were terminated by filtration through glass fiber filter paper (Schleicher and Schuell #32), using a 24 well cell harvester. The glass filter discs were washed 4 times with 4 ml of ice-cold 50 mM Na-K phosphate buffer (pH 7.4; containing 10% w/v polyethylene glycol for [^{125}I]HEAT binding) under vacuum suction. Tritium was determined by liquid scintillation counting after immersing the glass filter discs in scintillation cocktail overnight. In the experiments using [^{125}I]HEAT, the dpm values of the glass filter discs were counted with an automatic γ-counter. Three separate competitive experiments were performed for each of the competitors. Data were analyzed by a computerized non-linear regression program.

2.5. Statistical analyses

Experimental values are given as a mean ± S.E. Statistical significance was assessed by one-way analysis of variance and unpaired, two-tailed *t*-test, and $P < 0.05$ was considered significant.

3. Results

3.1. Assay conditions

The optimal pre-incubation interval for each radioligand was determined from two separate experiments. Each ex-

periment was performed with two different constant concentrations of radioligand as described in Section 2. These preliminary experiments demonstrated that pre-incubation interval required of [^{3}H]doxazosin and [^{125}I]HEAT is 20 min, and that pre-incubation was not required for [^{3}H]rauwolscine and [^{3}H]clonidine.

The optimal incubation interval for each radioligand was also determined from two separate experiments. Specific binding of [^{3}H]doxazosin, [^{125}I]HEAT, [^{3}H]rauwolscine and [^{3}H]clonidine reached a plateu at 40, 30, 40 and 40 min, respectively.

The optimal washing time for each radioligand was also determined from two separate experiments. A 4-min wash for [^{3}H]doxazosin, a 10-min wash for [^{125}I]HEAT and a 2-min wash for [^{3}H]rauwolscine and [^{3}H]clonidine after a brief rinse were considered optimal because the ratio of specific binding to non-specific binding was maximal under these washing conditions.

3.2. Binding studies on human prostate

The binding of [^{3}H]doxazosin, [^{125}I]HEAT, [^{3}H]-rauwolscine and [^{3}H]clonidine on slide-mounted tissue sections of the human prostate was consistently saturable and of high affinity. The equilibrium dissociation constants (K_d) and receptor densities (B_{max}) were determined from Scatchard analyses of the saturation experiments ($n = 8$). The mean K_d values for [^{3}H]doxazosin, [^{125}I]HEAT, [^{3}H]rauwolscine and [^{3}H]clonidine determined from slide-mounted tissue sections of the human prostate were 2.45 ± 0.21 nM, 105.4 ± 10.2 pM, 0.83 ± 0.06 nM and 8.06 ± 0.60 nM, respectively. The α_1-adrenoceptor density (mean B_{max} values) measured with [^{3}H]doxazosin and [^{125}I]HEAT were 1.19 ± 0.12 and 0.76 ± 0.11 fmol/mg wet weight, respectively. The α_2-adrenoceptor density (mean B_{max} values) measured with [^{3}H]rauwolscine and [^{3}H]clonidine were 0.54 ± 0.09 and 0.56 ± 0.11 fmol/mg wet weight, respectively. The proportions of specific binding to total binding of [^{3}H]doxazosin, [^{125}I]HEAT, [^{3}H]rauwolscine and [^{3}H]clonidine were 23, 58, 78 and 74%, respectively, at ligand concentrations approximating their K_d values.

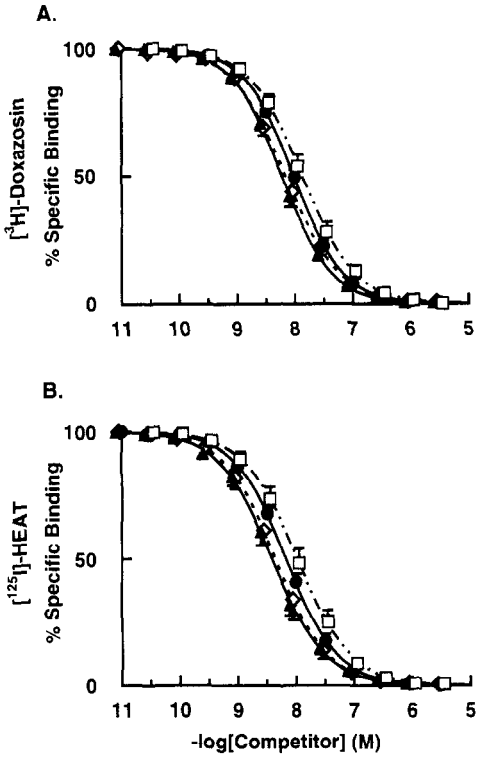

Fig. 2. Inhibition of specific binding of [^{3}H]doxazosin (A) and [^{125}I]HEAT (B) to α_1-adrenoceptors in the human prostate by α-adrenoceptor antagonists. Competitive binding experiments were performed on slide-mounted tissue sections in the presence of a constant final concentration of radioligand and varying concentrations of unlabeled *rac*-doxazosin (◇), *R*-doxazosin (●), *S*-doxazosin (▲) and *rac*-terazosin (□). The assays were performed in triplicate for each concentration of unlabeled ligand. Each point represents the mean ± S.E. of 6–7 experiments.

The affinities of *rac*-doxazosin, *R*-doxazosin, *S*-doxazosin and *rac*-terazosin for α_1- and α_2-adrenoceptors in the human prostate adenoma were determined using

Table 1
Potency of *rac*-, *R*- and *S*-doxazosin and *rac*-terazosin at the α_1- and α_2-adrenoceptors in the human prostrate

Compound-binding site	α_1 pK_i [N] (n_H)		α_2 pK_i [N] (n_H)		α_1/α_2
	[^{3}H]Doxazosin-binding site	[^{125}I]HEAT-binding site	[^{3}H]Rauwolscine-binding site	[^{3}H]Clonidine-binding site	Selectivity ratio
rac-Doxazosin	8.63 ± 0.07 [6] (0.98 ± 0.03)	8.60 ± 0.13 [6] (1.07 ± 0.04)	6.26 ± 0.07 [a] [6] (1.00 ± 0.02)	6.20 ± 0.10 [b] [6] (0.96 ± 0.02)	187–287
R-Doxazosin	8.55 ± 0.08 [7] (1.04 ± 0.02)	8.47 ± 0.05 [7] (1.00 ± 0.01)	6.47 ± 0.11 [a] [8] (0.90 ± 0.03)	6.54 ± 0.11 [a] [8] (0.99 ± 0.04)	107–140
S-Doxazosin	8.65 ± 0.08 [7] (1.02 ± 0.02)	8.61 ± 0.11 [7] (0.97 ± 0.02)	5.91 ± 0.08 [8] (0.90 ± 0.03)	5.94 ± 0.11 [8] (0.99 ± 0.05)	480–612
rac-Terazosin	8.45 ± 0.09 [6] (1.01 ± 0.02)	8.31 ± 0.11 [6] (1.01 ± 0.01)	5.80 ± 0.13 [6] (1.02 ± 0.02)	5.82 ± 0.14 [6] (0.96 ± 0.02)	331–510

Equilibrium competition experiments were performed as described in Section 2, on slide-mounted human prostatic tissue specimens. Estimates of equilibrium inhibition constants are shown as pK_i ($-\log K_i$) values and were determined by the non-linear regression analysis. N, number of experiments. n_H, pseudo-Hill coefficient. The values are expressed as mean ± S.E.

[a] $P < 0.05$ when compared to the pK_i value for *S*-doxazosin at the same radioligand-binding sites.

[b] $P < 0.05$ when compared to the pK_i value for *R*-doxazosin at the same radioligand-binding sites.

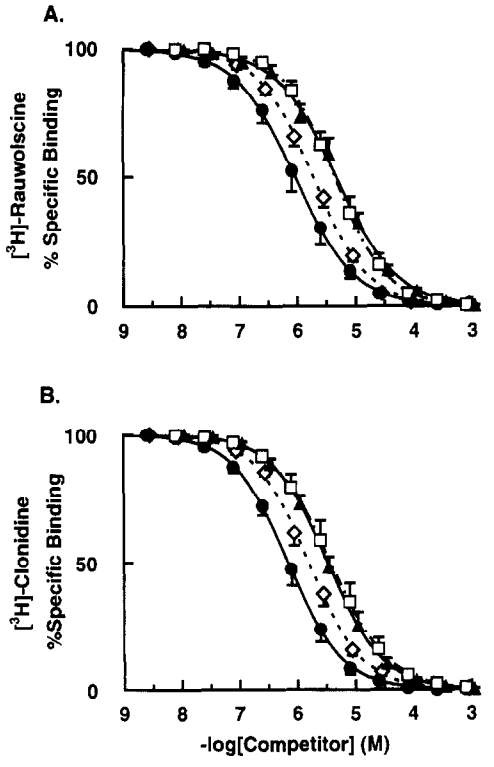

Fig. 3. Inhibition of specific binding of [^{3}H]rauwolscine (A) and [^{3}H]clonidine (B) to α_1-adrenoceptors in the human prostate by α-adrenoceptor antagonists. Competitive binding experiments were performed on slide-mounted tissue sections in the presence of a constant final concentration of radioligand and varying concentrations of unlabeled *rac*-doxazosin (◇), *R*-doxazosin (●), *S*-doxazosin (▲) and *rac*-terazosin (□). The assays were performed in triplicate for each concentration of unlabeled ligand. Each point represents the mean ± S.E. of 6–8 experiments.

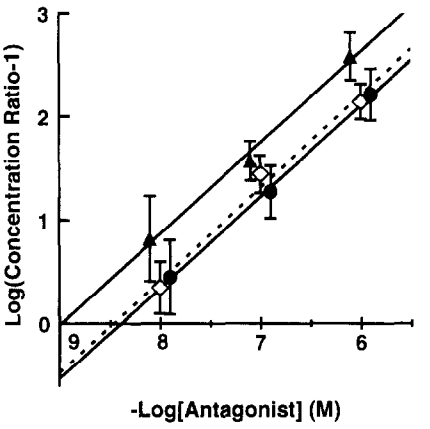

Fig. 4. Schild plots of the inhibition by α-adrenoceptor antagonists of the phenylephrine-induced contraction of the isolated human prostate. Strips of human prostate were incubated in the absence or presence (10^{-8}–10^{-6} M) of *rac*-doxazosin (◇), *R*-doxazosin (●) and *S*-doxazosin (▲), and the contractions induced by cumulative concentrations of phenylephrine (10^{-8}–10^{-2} M) were measured. Each point represents the mean ± S.E. of 4–5 experiments.

competitive inhibition experiments. The composite competitive inhibition plots for *rac*-doxazosin, its enantiomers and *rac*-terazosin in each radioligand binding are shown in Figs. 2 and 3. All the individual competition plots obtained using human prostate tissue were consistent with a single binding site model, and the pseudo-Hill coefficients were not different from unity (Table 1). The mean pK_i ($-\log K_i$) values determined from the individual competitive inhibition assays are summarized in Table 1. The pK_i values for the same competitor generated from two separate radioligands, i.e. [^{3}H]doxazosin and [^{125}I]HEAT for the α_1-adrenoceptors or [^{3}H]rauwolscine and [^{3}H]clonidine for the α_2-adrenoceptors, showed an excellent agreement. The mean pK_i values for all competitors tested in this study were consistently lower at the [^{3}H]rauwolscine- and [^{3}H]clonidine-binding sites compared with the [^{3}H]doxazosin- and [^{125}I]HEAT-binding sites. In the competitive binding studies with [^{3}H]doxazosin and [^{125}I]HEAT, no significant differences in the mean pK_i values were observed between *rac*-doxazosin, *R*-doxazosin and *S*-doxazosin. The competitive binding studies with [^{3}H]rauwolscine and [^{3}H]clonidine revealed differences in the mean pK_i values between *rac*-doxazosin, *R*-doxazosin and *S*-doxazosin. *S*-doxazosin ($pK_i = 5.91$–5.94) was slightly (3- or 4-fold), but significantly less potent in inhibition of [^{3}H]rauwolscine and [^{3}H]clonidine binding than *R*-doxazosin ($pK_i = 6.47$–6.54). From the K_i values obtained in [^{3}H]doxazosin or [^{125}I]HEAT binding and [^{3}H]rauwolscine or [^{3}H]clonidine binding, it was possible to compare the selectivity of individual antagonists for α_1-adrenoceptors (Table 1). *R*- and *S*-doxazosin displayed 107–140 and 480–612 times higher affinity for α_1-adrenoceptors than for α_2-adrenoceptors, respectively.

3.3. Isolated organ studies with human prostate

To characterize the functional properties of doxazosin and its enantiomers, the post-junctional α_1-adrenoceptor blocking potencies of these antagonists were evaluated in smooth muscle strips of the human prostate using phenylephrine as the selective α_1-adrenoceptor agonist. α_1-adrenoceptor stimulation by phenylephrine produced concentration-dependent increases in tension of the human prostate ($EC_{50} = 3.25 \pm 0.83 \times 10^{-6}$ M). *rac*-Doxazosin, *R*-doxazosin and *S*-doxazosin produced parallel and concentration-dependent shifts to the right of phenylephrine concentration-response curves without a reduction of the max-

Table 2
Potency of *rac*-, *R*- and *S*-doxazosin to inhibit phenylephrine-induced contraction of prostate smooth muscles

Compound	*N*	pA_2	Slope
rac-Doxazosin	5	8.43 ± 0.28	0.94 ± 0.07
R-Doxazosin	4	8.58 ± 0.40	0.88 ± 0.07
S-Doxazosin	4	8.75 ± 0.38	0.95 ± 0.09

Data shown as mean ± S.E. of the pA_2 and slope estimates with a number of experiments (*N*).

Table 3
Competition by *rac*-, *R*- and *S*-doxazosin and 5-methylurapidil for specific [^{3}H]prazosin binding at the cloned human α_1-adrenoceptors

Compound	pK_i (n_H)		
	α_{1a}	α_{1b}	α_{1d}
rac-Doxazosin	8.70 ± 0.03	9.14 ± 0.11	8.78 ± 0.16
	(0.96 ± 0.02)	(0.95 ± 0.03)	(1.02 ± 0.03)
R-Doxazosin	8.71 ± 0.16	9.07 ± 0.25	8.88 ± 0.08
	(0.99 ± 0.02)	(0.93 ± 0.03)	(0.98 ± 0.01)
S-Doxazosin	8.69 ± 0.06	9.03 ± 0.14	8.97 ± 0.09
	(1.01 ± 0.03)	(1.01 ± 0.04)	(1.01 ± 0.02)
5-Methylurapidil	8.81 ± 0.12 [a,b]	7.11 ± 0.07 [b,c]	7.93 ± 0.09 [a,c]
	(1.02 ± 0.01)	(0.97 ± 0.04)	(1.00 ± 0.02)

Equilibrium competition binding experiments using [^{3}H]prazosin were performed in membrane preparations from cultured rat-1 fibroblasts stably transfected with the cloned human α_{1a}- and α_{1b}-adrenoceptors. Estimates of equilibrium inhibition constants are shown as pK_i ($-\log K_i$) values and were determined by non-linear regression analysis. n_H, pseudo-Hill coefficient. The values are expressed as mean ± S.E. of 3 independent experiments.
[a] $P < 0.05$ vs. α_{1b}. [b] $P < 0.05$ vs. α_{1d}. [c] $P < 0.05$ vs. α_{1a}.

imal response. The slopes of the Schild plots were close to unity for all antagonists tested (Fig. 4, Table 2), suggesting that these antagonists competitively inhibited the contractile responses elicited by phenylephrine in the human prostate. The mean pA_2 values for *rac*-, *R*- and *S*-doxazosin are presented in Table 2. There were no significant differences in the pA_2 values between *rac*-doxazosin, *R*-doxazosin and *S*-doxazosin.

3.4. Binding studies on the cloned human α_1-adrenoceptor subtypes

Membrane preparations from rat-1 fibroblasts stably transfected with the cloned human α_1-adrenoceptor cDNAs exhibited saturable binding of [^{3}H]prazosin and [^{125}I]HEAT. The mean K_d values for [^{3}H]prazosin binding ($n = 3$) at the α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors were 0.36 ± 0.05, 0.38 ± 0.04 and 0.53 ± 0.05 nM, respectively, and the mean B_{max} values were 2.6 ± 0.7, 6.9 ± 1.9 and 1.8 ± 0.4 pmol/mg protein, respectively. The mean K_d values for [^{125}I]HEAT binding ($n = 3$) at the α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors were 85.3 ± 10.8, 96.5 ± 5.8 and 77.7 ± 8.1 pM, respectively, and the mean B_{max} values were 2.7 ± 0.03, 2.6 ± 0.7 and 1.7 ± 0.2 pmol/mg protein, respectively.

Table 4
Competition by *rac*-, *R*- and *S*-doxazosin and 5-methylurapidil for specific [^{125}I]HEAT binding at the cloned human α_1-adrenoceptors

Compound	pK_i (n_H)		
	α_{1a}	α_{1b}	α_{1d}
rac-Doxazosin	8.61 ± 0.08	8.83 ± 0.23	8.76 ± 0.11
	(1.02 ± 0.01)	(0.99 ± 0.02)	(0.95 ± 0.02)
R-Doxazosin	8.75 ± 0.18	8.89 ± 0.17	8.64 ± 0.22
	(1.01 ± 0.04)	(0.98 ± 0.03)	(0.99 ± 0.02)
S-Doxazosin	8.95 ± 0.07	8.69 ± 0.26	8.85 ± 0.14
	(1.02 ± 0.02)	(0.96 ± 0.06)	(0.97 ± 0.02)
5-Methylurapidil	8.75 ± 0.14 [a,b]	6.68 ± 0.10 [b,c]	7.91 ± 0.21 [a,c]
	(0.98 ± 0.04)	(0.94 ± 0.02)	(0.93 ± 0.05)

Equilibrium competition binding experiments using [^{125}I]HEAT were performed in membrane preparations from cultured rat-1 fibroblasts stably transfected with the cloned human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors. Estimates of equilibrium inhibition constants are shown as pK_i ($-\log K_i$) values and were determined by non-linear regression analysis. n_H, pseudo-Hill coefficient. The values are expressed as mean ± S.E. of 3 independent experiments.
[a] $P < 0.05$ vs. α_{1b}. [b] $P < 0.05$ vs. α_{1d}. [c] $P < 0.05$ vs. α_{1a}.

The affinities of *rac*-doxazosin, *R*-doxazosin, *S*-doxazosin and 5-methylurapidil for the cloned human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors was determined using competitive inhibition experiments. All the individual competition plots for the recombinant human α_1-adrenoceptor subtypes were consistent with a single binding site model, and the pseudo-Hill coefficients were all close to unity (Tables 3 and 4). The mean pK_i values determined from the individual competitive inhibition assays using [^{3}H]prazosin and [^{125}I]HEAT are summarized in Tables 3 and 4, respectively. In the competitive inhibition of either [^{3}H]prazosin or [^{125}I]HEAT binding, *rac*-doxazosin, *R*-doxazosin and *S*-doxazosin showed no significant differences in their mean pK_i values between the different α_1-adrenoceptor subtypes. In addition, the mean pK_i values for doxazosin and its enantiomers at the same α_1-adrenoceptor subtype were almost equivalent. In the competitive inhibition of both [^{3}H]prazosin and [^{125}I]HEAT binding, 5-methylurapidil showed marked differences in its potency to inhibit radioligand binding to the three different recombinant α_1-adrenoceptor subtypes. 5-Methylurapidil was 50–117-fold more potent at the α_{1a}-adrenoceptor ($pK_i = 8.75$–8.81) compared to the α_{1b}-adrenoceptor ($pK_i = 6.68$–7.11) and 7–8-fold more potent at the α_{1a}-adrenoceptor compared to the α_{1d}-adrenoceptor ($pK_i = 7.91$–7.93).

4. Discussion

In this study, we have evaluated the α-adrenoceptor antagonist activities of doxazosin and its optical isomers in vitro. Radioligand binding and isolated organ techniques were used to estimate the potency of *rac*-, *R*- and *S*-doxazosin at the α-adrenoceptors. One important observation from this study was that all these compounds exhibited significant selectivity for α_1-adrenoceptors over α_2-adrenoceptors. In the radioligand-binding studies using isolated human prostatic tissue, *rac*-, *R*-, *S*-doxazosin and *rac*-terazosin showed higher affinity for α_1-adrenoceptors than for α_2-adrenoceptors by two to three orders of magnitude. For α_1-adrenoceptor-binding affinity, no significant differences were observed between *rac*-doxazosin and its component enantiomers. These compounds were approximately equipotent compared to *rac*-terazosin in α_1-adrenoceptor antagonist activity. This is consistent, at least in

the case of the racemates, with the clinical profile of these agents in benign prostatic hyperplasia and hypertension.

The B_{max} value obtained by saturation analysis for [^{125}I]HEAT binding to human prostate tissue sections was lower than the value obtained for [^{3}H]doxazosin. This result might indicate that doxazosin could also interact with other sites in addition to α_1-adrenoceptor sites. Alternatively, this result may reflect intrinsic differences in the lipophilicity (tissue permeability) of the two ligands. Differences in ligand lipophilicity may also explain our uncommon finding of equivalent numbers of α_2-binding sites using an α_2-agonist ([^{3}H]clonidine) and an α_2-antagonist ([^{3}H]rauwolscine).

Rac-, *R*- and *S*-doxazosin all competitively antagonized the phenylephrine-induced contractile response of isolated human prostate in organ bath studies. Based on pA_2 values, doxazosin and its enantiomers were equipotent in blocking post-synaptic α_1-adrenoceptors in the human prostate. Thus, both the receptor-binding and functional studies indicated that there is no stereochemical specificity of doxazosin for the α_1-adrenoceptors in human prostate.

Recent comparative binding and functional studies have provided the most compelling evidence that the tension of human prostatic smooth muscle is mediated primarily by the α_{1A}-adrenoceptor (Forray et al., 1994; Marshall et al., 1995). The affinities of doxazosin and its enantiomers for the α_1-adrenoceptor subtypes were determined in radioligand-binding studies using the membrane preparations obtained from rat-1 fibroblasts expressing the human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes. *rac*-, *R*- and *S*-doxazosin all proved to be potent antagonists with balanced activity across all three cloned human α_1-adrenoceptor subtypes, while 5-methylurapidil showed selectivity for the human α_1-adrenoceptor subtypes with a rank order of potency of $\alpha_{1a} > \alpha_{1d} > \alpha_{1b}$. These results are consistent with earlier reports describing the potency of α-adrenoceptor antagonists at the cloned human and animal α_1-adrenoceptors (Forray et al., 1994; Kenny et al., 1994).

In the competitive radioligand-binding studies on the human prostate, it was noteworthy that the binding affinity of *S*-doxazosin for the α_2-adrenoceptors was slightly lower than that of *R*-doxazosin. In contrast to the α_1-adrenoceptor affinities, the α_2-adrenoceptor affinities of these two enantiomers were both very low, yet significantly different. Thus, the *S*-doxazosin exhibited a higher α_1/α_2 adrenoceptor selectivity ratio (480–612) in the human prostate as compared to the *R*-isomer (107–140), and *rac*-doxazosin showed intermediate selectivity between its component enantiomers. The α_1/α_2 adrenoceptor selectivity of *rac*-doxazosin and *rac*-terazosin in the present study was in agreement with the results in other studies (Kyncl et al., 1990; Lepor et al., 1988c,1990b).

The role of α_2-adrenoceptors in the human prostate is equivocal. The present study confirmed that the density of α_2-adrenoceptor is almost of the same order of magnitude as α_1-adrenoceptors in the human prostate. Some investigators have reported that the efficacy of a selective α_1-adrenoceptor antagonist prazosin in relieving the irritative symptoms is less than that of a non-selective α-adrenoceptor antagonist phenoxybenzamine (Hedlund et al., 1983; Caine, 1986). Radioligand-binding studies have suggested that there might be an increased α_2-adrenoceptor density in prostate adenomas obtained from men with symptomatic benign prostatic hyperplasia (Gup et al., 1990). Shapiro et al. (1987) have shown that canine prostatic urethral pressure is modulated by clonidine, a selective α_2-adrenoceptor agonist. Paradoxically, human prostate smooth muscle responds minimally to α_2-adrenoceptor agonists in vitro (Lepor et al., 1988a). The α_2-adrenoceptors in human prostate are presumably located pre-synaptically and inhibit the release of noradrenaline via an action on the autoreceptors. Accordingly, it does not seem that α_2-adrenoceptor blockade exerts relaxing effect on the human prostate smooth muscle. Moreover, non-selective α-adrenoceptor antagonists, such as phenoxybenzamine, have significant adverse events, and their therapeutic effect is possibly transient because the loss of α_2-adrenoceptor-mediated feedback control results in increased release of noradrenaline and the development of tolerance due to increased intrasynaptic noradrenaline levels. In the treatment of benign prostatic hyperplasia, therefore, an α_1-adrenoceptor antagonist with minimal activity at the α_2-adrenoceptor is clinically desirable. In this context, *rac*-doxazosin and its two optical enantiomers all represent desirable agents for the treatment of benign prostatic hyperplasia, because all these drugs would have virtually no effect on α_2-adrenoceptors at the plasma levels achieved at therapeutic doses.

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