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Alfuzosin, a selective α_1-adrenoceptor antagonist in the lower urinary tract

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1 Phenylephrine-induced contractions of rabbit isolated trigone and urethra were antagonized in a competitive manner by alfuzosin (pA_2 7.44 and 7.30, respectively) and prazosin.

2 The characteristics of [^{3}H]-prazosin binding to human prostatic adenoma tissue were evaluated. [^{3}H]-prazosin was potently displaced by α_1-adrenoceptor specific agents including alfuzosin, its (+)- and (−)-enantiomers and prazosin (IC_{50} 0.035, 0.023, 0.019 and 0.004 μM, respectively), but only weakly by α_2-adrenoceptor selective agents, for example, yohimbine (IC_{50} = 6.0 μM).

3 In the pithed rat, alfuzosin (0.03–0.3 mg kg^{-1}, i.v.) markedly inhibited pressor responses produced by the α_1-selective agonist, cirazoline but inhibited only slightly responses to the α_2-selective agonist, UK 14,304. Alfuzosin (1 mg kg^{-1}, i.v.) had minimal effects against responses mediated by stimulation of prejunctional α_2-receptors (UK 14,304-induced inhibition of sympathetic tachycardia).

4 In the anaesthetized cat, alfuzosin (0.001–1 mg kg^{-1}, i.v.) and prazosin (0.001–0.3 mg kg^{-1}, i.v.) produced dose-related inhibition of the increases in urethral pressure caused by stimulation of sympathetic hypogastric nerves. Prazosin was approximately 5 fold more potent than alfuzosin. When phenylephrine was employed to induce urethral and vascular α_1-mediated tone simultaneously, prazosin inhibited both stimuli with similar potency whereas alfusozin was 3–5 fold more potent against elevated urethral pressure. This functional uroselectivity of alfuzosin was more evident by the intraduodenal route, since doses of 0.03 and 0.1 mg kg^{-1} alfuzosin inhibited urethral pressure with minimal effects on arterial blood pressure.

5 Alfuzosin is a potent selective α_1-adrenoceptor antagonist in tissues of the lower urinary tract including the human prostate. This provides a pharmacological basis for its use in the treatment of benign prostatic hypertrophy.

Keywords: Alfuzosin; α_1-adrenoceptor antagonist; lower urinary tract; benign prostatic hypertrophy

Introduction

Benign prostatic hypertrophy (BPH), a condition which causes disturbance of micturition in a relatively high proportion of elderly men, has conventionally been treated by surgical resection of the enlarged prostate gland. However, more recently, alternative pharmacological treatment options have been developed, prominent amongst which is the use of selective α_1-adrenoceptor antagonists e.g. alfuzosin.

The outflow obstruction which accompanies abnormal prostate growth and which is reponsible for the observed urinary symptoms (difficulty in urination, incontinence, etc.) is considered to have two components, a static one related to physical compression of the urethra caused by prostatic hypertrophy and a dynamic component which derives from changes in smooth muscle tone within the prostate, bladder neck and urethra and is determined by the level of activation of the sympathetic nervous system (Caine, 1986). This dynamic and potentially reversible neurogenic factor contributes significantly to the total observed urethral obstruction (47%, Furuya *et al.*, 1982) and is probably responsible for the characteristic fluctuations in symptoms of voiding dysfunction experienced by BPH patients. A therapeutic strategy based on antagonism of the increased sympathetic drive to the lower urinary tract has added significantly to the recent management of BPH (Hieble & Caine, 1986).

It is known that three key tissues concerned in micturition in the context of BPH, i.e. the bladder base (trigone), the urethra and the prostate itself, contain smooth muscle with sympathetic innervation through the hypogastric nerve and contract in response to sympathetic activation. The receptor type primarily involved, and thus responsible for sympathetically-mediated bladder outflow obstruction in man, is the α_1-subtype. Although both α_1- and α_2-receptors have been identified within the human prostate, the latter appear to predominate within vascular and glandular tissue rather than in the smooth muscle-containing element, the stroma (James *et al.*, 1989). Hence, the selective α_1-agonist, phenylephrine, contracts human isolated prostatic strips (Kitada & Kumazawa, 1987) and responses to the sympathetic neurotransmitter noradrenaline are antagonized potently by selective α_1-antagonists but only weakly by α_2-antagonists (Hedlund *et al.*, 1985; Hieble *et al.*, 1985) demonstrating the functional importance of the α_1-subtype. Similarly, in human urinary bladder base and urethral preparations noradrenaline-induced contractions are α_1-mediated (Kunisawa *et al.*, 1985).

As a final test of this hypothesis, a number of clinical trials have demonstrated that α_1-antagonists, prazosin, alfuzosin and terazosin (Hedlund *et al.*, 1983; Lepor *et al.*, 1990; Jardin *et al.*, 1991) are effective at improving the symptoms of BPH. In a large placebo-controlled trial, alfuzosin, the subject of this paper, significantly reduced the incidence of obstructive and irritative symptoms and increased urinary flow rates (Jardin *et al.*, 1991). Although agents of this type are also capable of causing vascular effects (potentially undesirable in the context of BPH), alfuzosin was well-tolerated and its efficacy was maintained over a 6-month period. In this paper we describe the animal pharmacology of alfuzosin (N-[3-[(4-amino-6, 7-dimethoxyquinazolin-2-yl) methylamino] propyl] tetrahydro-2-furancarboxamide), a quinazoline derivative which differs structurally from prazosin in the absence of a piperidine moiety, with particular emphasis on its α_1-antagonist properties in the lower urinary tract *in vitro* and *in vivo*. Some of these data have already been presented in abstract form (Cavero *et al.*, 1984b; 1985; Pimoule *et al.*, 1989).

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Methods

Phenylephrine-contracted rabbit isolated trigone and urethra

Male rabbits (3–4 kg) were killed by cervical dislocation and strips (5 × 2 mm) of trigone muscle or urethral rings (5 mm), prepared as described by Ueda *et al.* (1984), were set up under 1 g tension in Krebs buffer containing (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7 and ascorbic acid 1.1. Propranolol (1 μM) was routinely included to eliminate possible effects mediated by stimulation of β-receptors. After a 2 h stabilization period, cumulative concentration-response curves to phenylephrine were determined before and after a 30 min incubation with alfuzosin or one of its optical isomers (0.03–3 μM) or prazosin (0.03–3 μM). Each preparation received only a single antagonist concentration or vehicle. Concentration-response curves to phenylephrine in the presence of antagonist or vehicle were expressed as a percentage of the maximal response of the control curve. The antagonist potency was evaluated by Schild analysis. For alfuzosin, (+)-alfuzosin and prazosin, pA_2 values and slopes were calculated from regression analysis of all available data points (log[DR − 1]). In the case of (−)-alfuzosin, because of limited data, a pA_2 estimate was calculated directly from the Schild equation.

Displacement of [^{3}H]-prazosin binding in human prostatic adenomyofibroma membrane preparations

Human prostatic adenomas were obtained during surgery for BPH. After dissection, the cranial and periurethral regions were stored at − 80°C until use. The tissue was homogenized in 30 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5) with the use of a Polytron homogenizer. The homogenate was washed by two successive centrifugations for 10 min at 38,000 *g*, with intermittent resuspension of the resulting pellet in an identical volume of fresh ice-cold Tris-HCl buffer. The final pellet was resuspended to 18 ml of Tris-HCl buffer g^{-1} original wet tissue weight and filtered over 2 × 4 layers of cheesecloth. A 900 μl aliquot of this membrane suspension, the equivalent of 50 mg original wet tissue weight, was incubated with [^{3}H]-prazosin (specific activity 30 Ci $mmol^{-1}$, DuPont de Nemours/NEN Products, Boston, MA, U.S.A.) in a final volume of 1 ml Tris-HCl buffer (pH 7.5) in the presence or absence of competing drugs. Following a 30 min incubation at 25°C, membranes were recovered from the homogenate by vacuum filtration over Whatman GF/B filters and washed with three 5 ml aliquots of ice-cold 50 mM Tris-HCl buffer. Filter-retained radioactivity was quantified by liquid scintillation spectrometry at an efficiency of 40–45%. Specific [^{3}H]-prazosin binding was defined as the decrease in the amount of filter-retained radioactivity after incubation in the presence of 100 μM phentolamine. The abilities of alfuzosin, prazosin and a variety of other reference compounds to displace [^{3}H]-prazosin binding were studied at a radioligand concentration of 0.5 nM and expressed as an IC_{50} value (concentration providing 50% inhibition of specific binding).

Functional responses to α_1- and α_2-adrenoceptor stimulation in pithed rats

Effects on postjunctional α_1- and α_2-adrenoceptor mediated vasoconstriction Male rats (250 g) were anaesthetized with sodium pentobarbitone (55 mg kg^{-1}, i.p.), pithed and placed under artifical respiration. Blood pressure and heart rate were measured from the carotid artery and recorded on a polygraph. The compounds were administered via a femoral vein. Pressor responses to cumulative doses of cirazoline (α_1-adrenoceptor agonist: 0.125 to 128 μg kg^{-1}, i.v.) were studied 5 min after a 5 min i.v. infusion of alfuzosin (0.03, 0.1 and 0.3 mg kg^{-1}), prazosin (0.01, 0.03 and 0.1 mg kg^{-1}) or solvent (isotonic NaCl solution: 0.4 ml kg^{-1}). In animals of a second group receiving alfuzosin (0.3 and 1.0 mg kg^{-1}), prazosin (0.1 and 0.3 mg kg^{-1}) or solvent intravenously during a 5 min period, the pressor effects of UK-14,304 (α_2-adrenoceptor agonist: 0.31 to 1280 μg kg^{-1}, i.v. dosed cumulatively) were studied 5 min after each treatment. Each agonist dose was injected when the maximal pressor effect of the preceding dose was reached. Dose-response curves were constructed and the doses of agonist producing a 50 mmHg increase in mean blood pressure (ED_{50} values) were determined in the presence of antagonist or solvent.

Effects on prejunctional α_2-adrenoceptor stimulation Animals were prepared as described above. The thoracic (C_6–T_5) spinal cord was continuously stimulated at low-frequency (0.1–0.2 Hz, 60 V, 0.5 ms) in order to induce a sustained increase in heart rate of about 100 beats min^{-1}. The metal pithing rod was varnished except for 1 cm approximately 6 cm from the caudal extremity. Cumulative doses of the prejunctional α_2-adrenoceptor agonist UK-14,304 (0.125 to 256 μg kg^{-1}, i.v.) were administered; each dose was injected when the maximal effect of the previous one had been attained. The inhibitory dose-response curve to UK-14,304 on experimental sympathetic tachycardia was determined for animals pretreated with alfuzosin (1.0 mg kg^{-1}), prazosin (0.3 mg kg^{-1}), idazoxan (0.3 mg kg^{-1}) or solvent (isotonic NaCl); these drugs were administered by a 5 min intravenous infusion. Results are expressed as mean ± s.e.mean and significance was determined by Student's *t* test for unpaired series.

Measurement of urethral pressure elevated by sympathetic nerve stimulation or phenylephrine infusion in anaesthetized cats

Sympathetic nerve stimulation Adult cats of either sex weighing 2.5–3.5 kg were anaesthetized with sodium pentobarbitone (42 mg kg^{-1}, i.p. + 6 mg h^{-1}, i.v. throughout the duration of the experiment) and artificially ventilated with room air. A catheter was introduced into the brachial artery for measurement of arterial pressure and heart rate using a cardiotachometer triggered by the aortic pulse wave. A catheter was introduced into the bladder initially at the level of the trigone and subsequently advanced into the first half of the urethra for measurement of intraurethral pressure after ligature of the bladder neck. Catheters were placed in the cephalic and femoral veins for drug administration. Cardiovascular and urological parameters were recorded with a polygraph. The right hypogastric nerve was isolated and prepared for electrical stimulation (5 V; 20 Hz; 2 ms; 15 s). In order to eliminate autonomic neural activity other than that evoked through α-adrenoceptors, all animals were treated with chlorisondamine (0.5 mg kg^{-1}, i.v.), atropine (0.5 mg kg^{-1}, i.v.) and propranolol (0.75 mg kg^{-1}, i.v.). Alfuzosin was administered in cumulative doses (1–1000 μg kg^{-1}, i.v., using dose steps of 1, 3, 10, 20, 55, 100, 300, 500 and 1000 μg kg^{-1} each dose being given over 5 min), and its effects upon smooth muscle contraction triggered by electrical stimulation of the hypogastric nerve were studied 5 min after each dose. Comparisons were made with prazosin (cumulative doses: 1–300 μg kg^{-1}, i.v. using the same dose steps for alfuzosin) or solvent (5% glucose). Previous studies had shown that the activity of alfuzosin or prazosin was maximal 5 min after treatment. The doses of α-antagonist necessary to cause 50% inhibition of urethral hypertonia were determined (ED_{50}).

In a separate series of experiments alfuzosin (0.1–3 mg kg^{-1}) was administered via the intraduodenal route and its effects on elevated intraurethral pressure monitored during the 3 h period which followed drug administration.

Phenylephrine infusion Anaesthetized male cats (3–4.5 kg) were prepared for recording blood pressure and urethral pressure as described above. Urethral pressure and arterial

blood pressure were elevated in a sustained fashion by continuous i.v. infusion of phenylephrine (9–12 $\mu g\ kg^{-1}\ min^{-1}$). Once the preparation was stable, alfuzosin (1–1000 $\mu g\ kg^{-1}$, i.v.), prazosin (1–1000 $\mu g\ kg^{-1}$, i.v.) or vehicle (5% glucose) was administered cumulatively, doses being added once the effects of the previous dose had reached maximum. The ED_{50} and ED_{80} values (doses of antagonist necessary to produce 50% or 80% inhibition of urethral pressure and blood pressure) were calculated by linear regression analysis. This procedure involved fitting a straight line to data points in the linear region of each dose-response curve, checking for linearity, deriving the appropriate regression equation and using this equation to calculate the doses which corresponded to each of the set response levels (50% or 80% inhibition). In a separate series of experiments using the same phenylephrine infusion protocol, alfuzosin was administered as a single intraduodenal dose (0.03 or 0.1 $mg\ kg^{-1}$) and its effects monitored for 60 min following administration.

Drugs used

Alfuzosin hydrochloride, (+)-alfuzosin hydrochloride, (−)-alfuzosin hydrochloride, prazosin hydrochloride, cirazoline, idazoxan hydrochloride and UK-14,304 (5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline) were synthesized in the Department of Chemistry, Synthélabo Recherche. Sources of other drugs used were as follows: phenylephrine hydrochloride, noradrenaline bitartrate, yohimbine hydrochloride, papaverine hydrochloride (Sigma); chlorisondamine chloride, phentolamine hydrochloride (Ciba-Geigy); atropine sulphate (Prolabo); propranolol (ICI); phenoxybenzamine hydrochloride (SK&F); pentobarbitone sodium (Sanofi).

Results

Rabbit isolated trigone and urethra

The selective α_1-adrenoceptor agonist, phenylephrine, caused concentration-dependent contractions of the isolated trigone and urethra and was virtually equipotent in both preparations. The pD_2 (− log molar EC_{50}) for phenylephrine was 4.9 ± 0.07 in the urethra ($n = 26$) and 5.2 ± 0.07 in the trigone ($n = 26$).

Alfuzosin (0.1–3 μM) or prazosin (0.03–3 μM) caused significant parallel rightward shifts of the phenylephrine concentration-response curve in both trigone and urethra. The maximum contractile response to phenylephrine was unaffected by the presence of alfuzosin or prazosin, except at the highest concentration of each where a reduction of maximum of approximately 20%–30% was observed.

The α_1-adrenoceptor antagonist potencies of alfuzosin and prazosin were evaluated by Schild analysis and the pA_2 values are shown in Table 1. The slopes of the Schild plots were not significantly different from unity, indicating that both drugs were apparently competitive antagonists against phenylephrine-induced responses in these preparations.

Table 2 Pharmacological profile of [^{3}H]-prazosin binding to cranial human prostate adenoma

Drug	IC_{50} (μM)
Prazosin	0.004 ± 0.001
Phentolamine	0.018 ± 0.001
Alfuzosin	0.035 ± 0.008
(+)-Alfuzosin	0.023 ± 0.002
(−)-Alfuzosin	0.019 ± 0.006
Phenoxybenzamine	0.038 ± 0.016
Idazoxan	3.5 ± 1.0
Yohimbine	6.0 ± 2.0
Phenylephrine	7.0 ± 1.5
UK 14,304	36 ± 14
Propranolol	37 ± 9
Papaverine	60 ± 15
Noradrenaline	1.1 ± 0.46

Drug concentrations producing a 50% inhibition of specific [^{3}H]-prazosin binding (IC_{50}) are shown as the mean ± s.e. of at least three experiments.

Both enantiomers of alfuzosin also inhibited responses to phenylephrine in trigone and urethra with a potency similar to that of racemic alfuzosin (Table 1).

[^{3}H]-prazosin binding in human prostatic adenomyofibroma tissue

Using a radioligand concentration-range of 0.01–10 nM non-specific [^{3}H]-prazosin binding, determined in the presence of 100 μM phentolamine, increased linearly as a function of the radioligand concentration. Specific [^{3}H]-prazosin binding, in contrast, increased non-linearly with the radioligand concentration, and at 0.8 nM [^{3}H]-prazosin specific binding represented approximately 55% of the total amount of [^{3}H]-prazosin retained by the filter. Non-linear regression analysis indicated that the data are adequately described by a model of [^{3}H]-prazosin binding to a single homogeneous class of binding sites ($K_D = 0.17 \pm 0.02$ nM) with a maximal binding capacity (B_{max}) of 41 ± 3 $fmol\ mg^{-1}$ protein ($n = 18$).

As shown in Table 2, [^{3}H]-prazosin binding was inhibited with high affinity by the specific α_1-adrenoceptor antagonists, prazosin, alfuzosin and both enantiomers of alfuzosin but with only low affinity by idazoxan, a selective α_2-antagonist. The relative potencies of various other commonly used adrenoceptor ligands are shown in Table 2 and confirm that [^{3}H]-prazosin labels the α_1-adrenoceptor in this model.

α_1 and α_2-adrenoceptor mediated responses in pithed rats

Effects on postjunctional α_1- and α_2-adrenoceptor mediated vasoconstriction Cirazoline caused dose-related increases in mean blood pressure with an ED_{50} value of 0.9 ± 0.05 $\mu g\ kg^{-1}$, i.v., in the presence of solvent. Figure 1 shows that both alfuzosin and prazosin produced marked parallel right-

Table 1 Antagonist potency of alfuzosin, its enantiomers and prazosin against phenylephrine-induced contractions of the rabbit trigone and urethra preparations

	Trigone			*Urethra*		
Antagonist	pA_2	*Slope*	n	pA_2	*Slope*	n
Alfuzosin	7.44 (7.25–7.69)	0.87	24	7.30 (7.04–7.75)	0.78	17
(+)-Alfuzosin	7.74 (7.44–8.55)	0.76	7	7.77 (7.53–8.24)	0.74	8
(−)-Alfuzosin	7.55 (7.47–7.63)	–	5	7.69 (7.52–7.86)	–	5
Prazosin	7.88 (7.33–8.43)	1.13	9	7.96 (7.11–8.81)	1.17	7

pA_2 values are shown with 95% confidence limits. None of the slopes differed significantly from 1. n = number of dose-ratios.

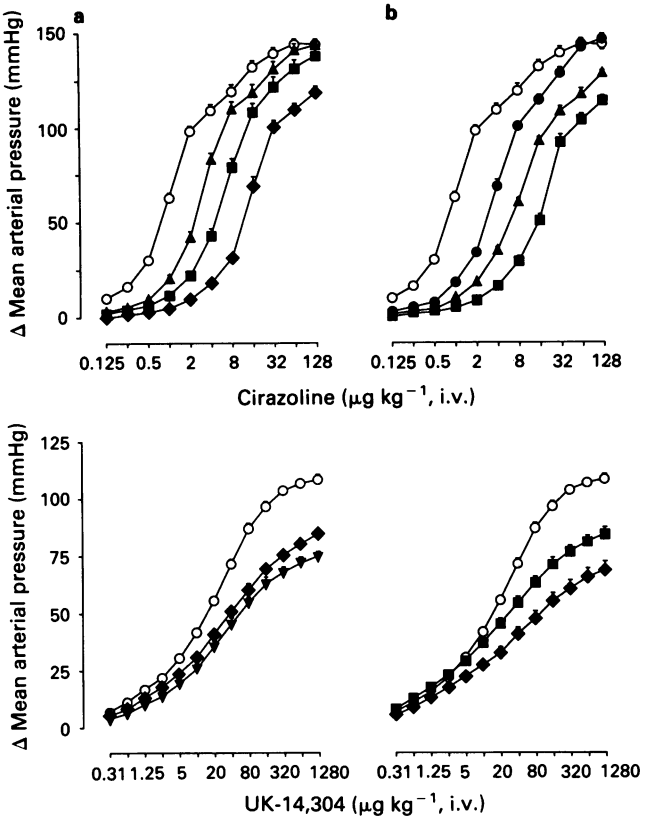

Figure 1 Effects of (a) alfuzosin and (b) prazosin on pressor responses induced by the selective α_1-agonist cirazoline (upper panel) and the selective α_2-agonist UK 14,304 (lower panel) in pithed rats. Responses were obtained under control conditions (○) and following antagonist doses of 0.01 (●), 0.03 (▲), 0.1 (■), 0.3 (◆) or 1 mg kg^{-1}, i.v. (▼). Values shown are means and s.e.mean (n = 5–7).

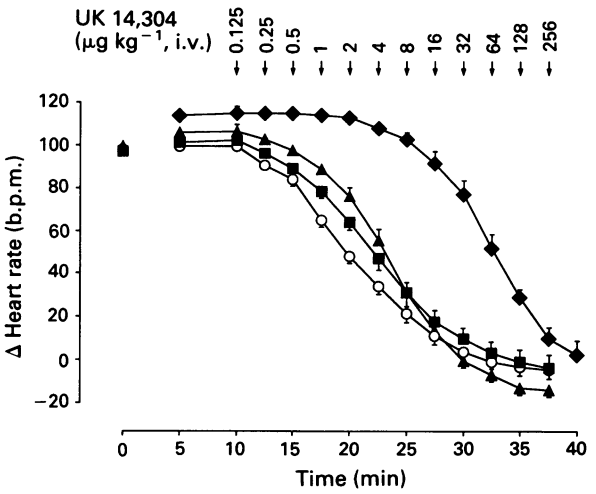

Figure 2 Effects of alfuzosin 1.0 mg kg^{-1}, i.v. (▲), prazosin 0.3 mg kg^{-1}, i.v. (■), idazoxan 0.3 mg kg^{-1}, i.v. (◆) and vehicle (○) on the inhibitory dose-response curve to the selective α_2-agonist, UK 14,304, against tachycardia produced by sustained stimulation of sympathetic nerves in the pithed rat. Electrical stimulation of thoracic spinal cord was performed continuously between − 10 and + 40 min. UK 14,304 was administered as cumulative i.v. bolus doses (0.125–256 μg kg^{-1}) at 2.5 min intervals commencing at + 10 min. Antagonists or vehicle were administered between 0 and + 5 min. Values shown are means ± s.e.mean (n = 4–5).

ward displacements of the cirazoline curve in a dose-related fashion at all antagonist doses tested. The maximum response to cirazoline appeared unchanged except possibly at the highest dose of each antagonist. The minimum effective antagonist doses were $\leqslant$ 0.03 mg kg^{-1}, i.v. for alfuzosin and $\leqslant$ 0.01 mg kg^{-1}, i.v. for prazosin. To quantitate antagonist potency we have calculated in each case the mean antagonist dose necessary to produce a 10 fold increase in the ED_{50} value of cirazoline (DR_{10} value). The DR_{10} values for alfuzosin and prazosin were 0.22 mg kg^{-1} and 0.043 mg kg^{-1}, respectively indicating that alfuzosin was approximately 5 times less potent than prazosin.

To establish the selectivity of alfuzosin and prazosin for postjunctional α_1-receptors, high concentrations of each antagonist were tested against pressor responses produced by the α_2-selective agonist, UK-14,304 (Figure 1). At antagonist doses which exceeded the DR_{10} doses versus cirazoline, alfuzosin (0.3–1 mg kg^{-1}, i.v.) and prazosin (0.1–0.3 mg kg^{-1}, i.v.) produced only slight antagonism of the UK-14,304 response curve with some depression of maximum. Dose ratios (DR) calculated against UK-14,304 using the ED_{50} values were 2.6 ± 0.3 and 3.6 ± 0.4 for alfuzosin at 0.3 and 1.0 mg kg^{-1} and 2.2 ± 0.3 and 6.7 ± 1.0 following prazosin 0.1 and 0.3 mg kg^{-1}, respectively.

Effects on cardiac prejunctional α_2-adrenoceptor stimulation Low frequency continuous sub-maximal stimulation of sympathetic nerves innervating the cardiac region caused a sustained increase in heart rate (101 ± 1 beats min^{-1}, n = 19) which was stable for the duration of the experimental procedure.

Cumulative administration of UK-14,304 produced a dose-related inhibition of sympathetically-induced tachycardia which was complete at a dose between 32–64 μg kg^{-1}, i.v. Figure 2 shows that the selective α_2-antagonist idazoxan (0.3 mg kg^{-1}, i.v.) elevated heart rate by approximately 15 beats min^{-1} and markedly antagonized the anti-accelerator responses to UK-14,304, the agonist dose-response curve being displaced to the right approximately 20 fold. In contrast, neither prazosin (0.3 mg kg^{-1}, i.v.) nor alfuzosin (1 mg kg^{-1}, i.v.) significantly altered responsiveness to UK-14,304. ED_{50} values for UK-14,304 were 1.27 ± 0.23 μg kg^{-1} (vehicle), 2.15 ± 0.4 μg kg^{-1} after prazosin, 2.25 ± 0.39 μg kg^{-1} after alfuzosin and 27.11 ± 4.3 μg kg^{-1} after idazoxan. Only the idazoxan value was significantly different from vehicle ($P < 0.05$).

Elevated urethral pressure in the anaesthetized cat

Sympathetic nerve stimulation Stimulation of the hypogastric sympathetic nerves caused reproducible increases in urethral pressure within the range of 30–45 cmH_2O which did not differ significantly between groups of animals. None of the treatments significantly changed basal blood pressure during the course of the experiments. Pre- and post-treatment values in mmHg in the three groups were, vehicle 74 ± 4, 72 ± 2, alfuzosin 76 ± 6, 70 ± 5, prazosin 68 ± 2, 64 ± 3.

Intravenous administration of both alfuzosin and prazosin (Figure 3) produced dose-related inhibition of sympathetically mediated increases in urethral pressure, whereas the solvent was without significant effect. ED_{50} values for alfuzosin and prazosin were 0.077 ± 0.003 mg kg^{-1} and 0.016 ± 0.001 mg kg^{-1}, respectively, showing that alfuzosin was approximately 5 times less potent than prazosin. The α_2-antagonist, yohimbine (0.3 mg kg^{-1}, i.v.) did not modify the urethral response to sympathetic nerve stimulation (data not shown).

When given by the intraduodenal route alfuzosin (0.1–3 mg kg^{-1}) produced sustained dose-related inhibition of the increases in urethral pressure (Figure 3) with an ED_{50} of 0.36 ± 0.06 mg kg^{-1}. The inhibitory effect was rapid in onset, reached maximum within 45 min–1 h after dosing and was still evident at 3 h.

Phenylephrine infusion The infusion of phenylephrine produced sustained increases in arterial blood pressure and

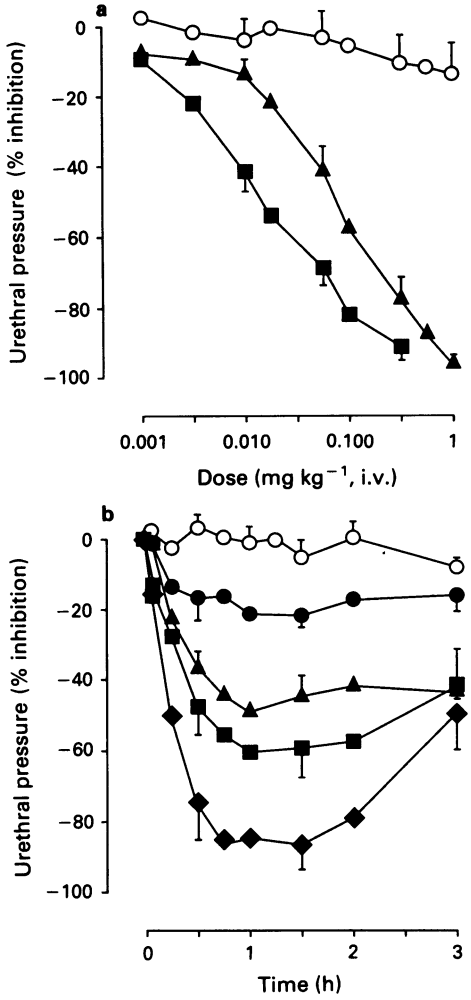

Figure 3 (a) Effects of cumulative i.v. bolus doses of alfuzosin (▲), prazosin (■) or vehicle (○) on the increase in urethral pressure induced by electrical stimulation of sympathetic hypogastric nerves in the anaesthetised cat. Values shown are means ± s.e.mean (n = 6–13). (b) In the same model, time course of inhibition of urethral pressure produced by single intraduodenal administrations of alfuzosin 0.1 (●), 0.3 (▲), 1 (■) or 3 mg kg^{-1} (◆), or vehicle (○). Values shown are means ± s.e.mean (n = 4–5).

urethral pressure. The respective increases observed for each parameter were 127.3 ± 4.4 mmHg and 28.1 ± 2.7 cmH_2O (n = 15, i.v. series) and 126.5 ± 2.9 mmHg and 30.4 ± 1.8 cmH_2O (n = 16, intraduodenal series).

In the i.v. antagonist studies the solvent did not lead to any significant changes in urethral or blood pressures (Figure 4). However, alfuzosin and prazosin caused dose-related decreases in both parameters. Whereas prazosin was approximately equipotent against urethral and vascular stimuli, alfuzosin demonstrated approximately 3–5 fold higher potency against elevated urethral pressure (ED_{50} and ED_{80} response levels) when compared with the arterial pressure response. In addition, alfuzosin but not prazosin inhibited urethral pressure by >100% i.e. to a greater extent than the original stimulation evoked by phenylephrine. The ED_{50} values (with 95% confidence limits) for urethral pressure were 0.031 (0.028–0.033) mg kg^{-1} and 0.014 (0.011–0.016) mg kg^{-1}, and for arterial pressure, 0.079 (0.068–0.090) mg kg^{-1} and 0.018 (0.016–0.020) mg kg^{-1}, for alfuzosin and prazosin respectively. The corresponding ED_{80} values for urethral pressure were 0.106 (0.101–0.110) mg kg^{-1} and 0.053 (0.041–0.081) mg kg^{-1}, and for arterial pressure, 0.465 (0.362–0.660) mg kg^{-1} and 0.091 (0.071–0.128) mg kg^{-1}, for alfuzosin and prazosin, respectively. Thus alfuzosin was only two times less potent than prazosin against urethral hypertonia but was five times weaker than prazosin for effects on arterial pressure. Statistical analysis indicated that the preferential effect of alfuzosin on urethral pressure, relative to arterial pressure, was significant (P<0.01, three way analysis of variance).

For intraduodenal studies with alfuzosin (Figure 5) the duration of the experiments was limited to the 60 min period following dosing due to a tendency for urethral and arterial pressures to decline in the control group. Alfuzosin (0.03 and 0.1 mg kg^{-1} intraduodenally) caused a rapid and marked decrease in elevated urethral pressure reaching a plateau 30–40 min after dosing. In contrast, the changes in arterial pressure following 0.03 mg kg^{-1} alfuzosin were similar to those observed in the control group and, at the higher dose (0.1 mg kg^{-1}), alfuzosin produced only slight inhibition. Hence, given by the intraduodenal route alfuzosin demonstrated a potent and relatively selective inhibition of phenylephrine-induced elevation of urethral pressure.

Discussion

The data presented show conclusively that alfuzosin is a potent competitive antagonist of α_1-adrenoceptors in the

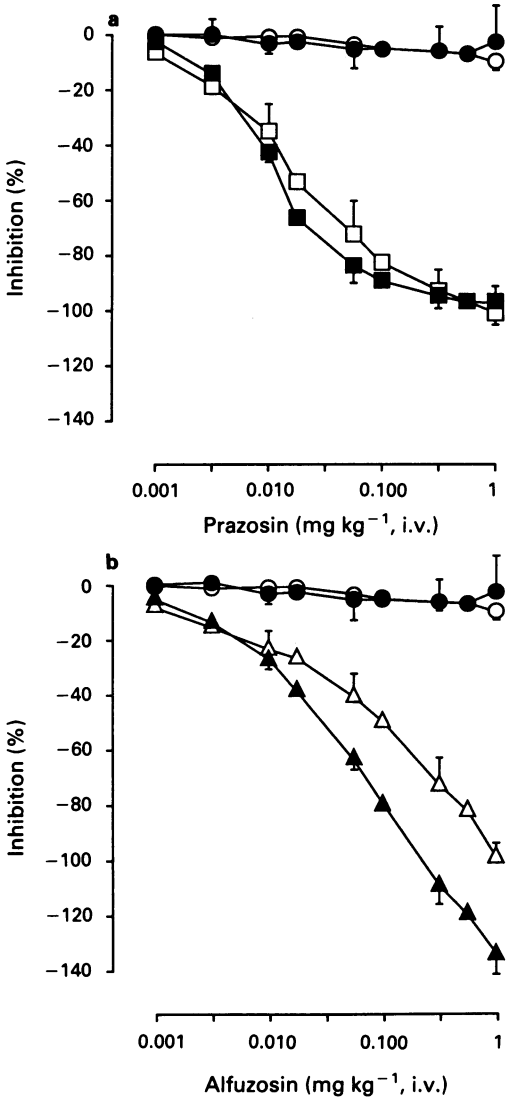

Figure 4 Anaesthetized cat preparations having arterial blood pressure (open symbols) and urethral pressure (filled symbols) elevated by continuous i.v. infusion of the α_1-agonist, phenylephrine. Percentage inhibition of these parameters by cumulative i.v. bolus doses of prazosin (■, □, a), alfuzosin (▲, △, b) or vehicle (●, ○). Values shown are means ± s.e.mean (n = 4–6).

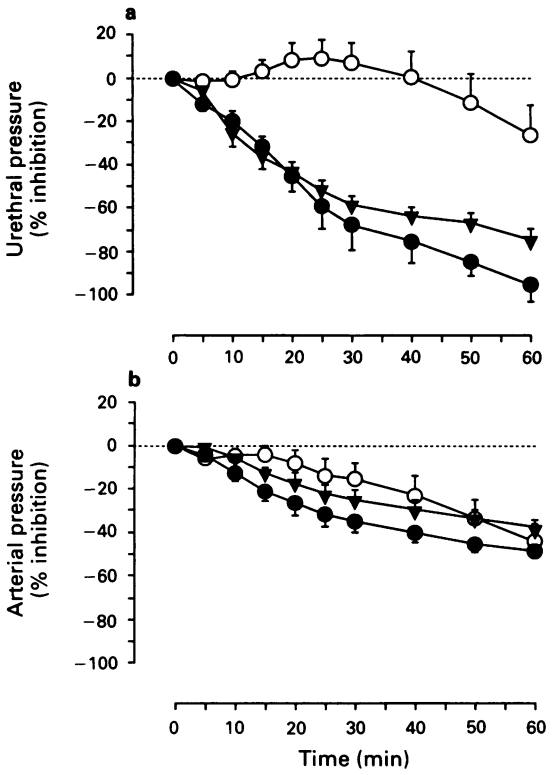

Figure 5 Anaesthetized cat preparations having urethral pressure (a) and arterial blood pressure (b) elevated by continuous i.v. infusion of the α_1-agonist, phenylephrine. Time course of the inhibitory effects of these two parameters of intraduodenal administration of alfuzosin 0.03 mg kg^{-1} (▼), 0.1 mg kg^{-1} (●) or its vehicle (○). Values shown are means ± s.e.mean ($n = 5-6$).

lower urinary tract. In this respect, its profile was similar to that of prazosin although alfuzosin was in general slightly less potent. The affinity of alfuzosin for α_1-receptors in the lower urinary tract is of the same order as that reported for α_1-receptors in other isolated tissues, e.g. rabbit pulmonary artery: pA_2 7.47 versus phenylephrine, displacement of [^{3}H]-prazosin binding in rat cerebral cortex: IC_{50} 15 nM (Cavero *et al.*, 1984a). Ligand binding and functional studies demonstrate that the α_1-antagonist properties of alfuzosin reside equally in its two enantiomers. This absence of stereoselectivity distinguishes alfuzosin from certain other α_1-antagonists, for example IP66 (Manzini *et al.*, 1991) and YM-12617 (Yamada *et al.*, 1987).

The separation of α-adrenoceptors into α_1- and α_2-subtypes (Langer, 1974) and the subsequent extension of this classification to allow for the presence of α_2-receptors at both postjunctional and prejunctional sites (Langer *et al.*, 1980) is well recognised. Taken in the context of the treatment of BPH the α_1/α_2 selectivity ratio may be a significant factor in determining clinical utility amongst α-antagonists. Hence, since functional sympathetic tone to the smooth muscle of the bladder trigone, urethra and prostatic stroma in man is largely or exclusively mediated via α_1-receptors (Kunisawa *et al.*, 1985; Hieble *et al.*, 1985) there would be little additional advantage to be gained by simultaneously blocking postjunctional α_2-receptors. Furthermore, antagonism of prejunctional α_2-receptors on sympathetic nerve terminals would inhibit negative feedback by neurotransmitter noradrenaline thus augmenting neurotransmitter release. This would tend to counteract the beneficial effects of postjunctional α_1-blockade and exacerbate sympathetically mediated bladder outflow obstruction. Although these theoretical arguments favour the use of an α_1-selective antagonist in BPH, it has to be recognised that the non-selective α-blockers, phentolamine and phenoxybenzamine, have been reported to show clinical efficacy in this condition although neither is a drug of choice (Christmas & Kirby, 1991). The present results demonstrate that, in *in vivo* functional studies, alfuzosin shows a high selectivity for α_1- versus α_2-receptors. In a direct comparison of its ability to block postjunctional α_1- and α_2-mediated responses in the pithed rat, alfuzosin was approximately 30 fold more potent as an antagonist of cirazoline-induced pressor responses by comparison with those produced by the α_2-selective agonist UK-14,304. In addition, since the α_2-selectivity of UK-14,304 is not absolute (Beckeringh *et al.*, 1984) it is conceivable that the weak inhibitory effects of alfuzosin, which were most evident at high doses of UK-14,304, actually reflect an α_1-agonist component in the vasopressor response. Prazosin, considered to have a large selectivity margin between α_1- and α_2-receptors, showed a similar profile in this model. Prejunctional α_2-antagonist properties were evaluated by testing alfuzosin and prazosin against UK-14,304-induced antiaccelerator effects in the presence of a tachycardiac response to cardiac sympathetic nerve stimulation. Although this response was highly sensitive to the α_2-antagonist idazoxan, alfuzosin was without significant inhibitory effect at 1 mg kg^{-1}, i.v., a dose 300 times greater than its threshold α_1-antagonist dose based on inhibition of urethral hypertonia in anaesthetized cats using phenylephrine as agonist.

Using [^{3}H]-prazosin as a selective radioligand for the α_1-adrenoceptor, we have identified high affinity [^{3}H]-prazosin recognition sites in the cranial region of the human prostatic adenoma that display the characteristics expected of the α_1-adrenoceptor. Thus, [^{3}H]-prazosin binding was potently inhibited by the α_1-adrenoceptor antagonists, alfuzosin, prazosin and phentolamine, with IC_{50} values in the low nanomolar range. In contrast, the α_2-adrenoceptor antagonists, idazoxan and yohimbine, or the β-adrenoceptor antagonist, propranolol, only affect [^{3}H]-prazosin binding with IC_{50} values in the micromolar range. Our data thus confirm other reports (Lepor & Shapiro, 1984; Yamada *et al.*, 1987) demonstrating specific α_1-adrenoceptor binding sites in the human prostate, the 'target tissue' in BPH, and demonstrates that alfuzosin shows high affinity for such sites. There is also evidence that the number of α_1-receptors in the abnormal prostate (BPH) is higher than that found in normal prostatic tissue (Yamada *et al.*, 1987) possibly as a direct result of hypertrophy of the muscular stroma, although other studies do not support this (Gup *et al.*, 1990). Studies in isolated prostate smooth muscle preparations demonstrate that the maximum contractile response to the α_1-agonist, phenylephrine, is doubled in tissues from BPH patients (Kitada & Kumazawa, 1987). A recent report shows that alfuzosin-displaceable [^{125}I]-HEAT binding sites (α_1-receptor) are exclusively associated with the muscular stroma of the prostate and are globally elevated in sections from prostatic adenomas as compared with normal prostate tissue (Benavides *et al.*, 1991).

In vivo experiments in the anaesthetized cat confirm the α_1-antagonist properties of alfuzosin. The compound produced dose-related and complete inhibition of the rise in urethral pressure resulting from postganglionic stimulation of hypogastric sympathetic nerves. This can be considered to represent an animal model of the dynamic sympathetic constriction of urethral smooth muscle which is regarded as a contributory factor in the micturition disorders which characterize BPH. Furthermore, as in man, the urethral hypertonic response in the cat is mediated exclusively by stimulation of α_1-receptors since it was completely inhibited by prazosin but unaffected by the α_2-antagonist yohimbine. Alfuzosin was also effective by the intraduodenal route producing dose-related and prolonged inhibition of urethral responses.

We were interested to establish the relationship between the ability of alfuzosin to inhibit sympathetically-mediated increases in urethral tone and its effect on sympathetically-mediated vasoconstriction *in vivo* since both responses involve activation of postjunctional α_1-receptors. Such comparsions may be relevant to the therapeutic margin of α_1-antagonists

in BPH with respect to unwanted vascular effects (e.g. orthostatic hypotension). In view of the technical difficulties involved in stimulating both vascular and urethral sympathetic nerves simultaneously in the same animal in an equal fashion, we designed, as an alternative, experiments using i.v. infusion of the α_1-agonist phenylephrine. Phenylephrine infusion caused sustained elevations of urethral pressure and arterial blood pressure. The dose of phenylephrine was selected with a view to producing responses of the two parameters in question which were as large as possible although still submaximal and within the linear region of the dose-response curve. Arterial pressure was slightly more sensitive to phenylephrine than urethral pressure ($\simeq$80% and $\simeq$65% of their respective maxima). These α_1-agonist urethral and vascular stimuli were equally sensitive to prazosin which produced dose-related and complete inhibition of both parameters with similar potency. In addition, the inhibitory potency of prazosin against phenylephrine induced increases in urethral pressure was virtually identical to its potency in inhibiting urethral hypertonic response to sympathetic nerve stimulation (ED_{50} values 0.014 mg kg^{-1}, i.v. and 0.016 mg kg^{-1}, i.v., respectively).

Alfuzosin also caused dose-related inhibition of phenylephrine-induced responses consistent with its α_1-antagonist properties; however, in contrast to prazosin, certain observations did not fit the expected pattern. In the first place, alfuzosin was moderately but significantly more potent in inhibiting the urethral hypertonic response compared with the pressor response. This separation between urethral and vascular indices in the presence of phenylephrine appeared at low doses of alfuzosin $\simeq$20 μg kg^{-1} and increased progressively with dose to give a uroselectivity ratio of $\simeq$5 at the 80% response level. Interestingly, alfuzosin but not prazosin decreased urethral pressure by >100%, i.e. by an amount greater than the total tone induced with phenylephrine. Alfuzosin was approximately three times more potent against urethral responses to phenylephrine than against those to sympathetic nerve stimulation. The ability of alfuzosin to inhibit selectively the urethral response to phenylephrine was even more evident when the compound was given by the intraduodenal route. Although the stability of this preparation did not allow the full time course of each response to be monitored, at maximum effect (achieved within 30–40 min of dosing) alfuzosin caused a marked inhibition of elevated urethral pressure but had little effect on the phenylephrine pressor response. It is noteworthy that these effects were observed at relatively low doses of alfuzosin (30 and 100 μg kg^{-1} intraduodenally) and that alfuzosin was approximately 10 times more potent in this series of experiments than when tested against nerve stimulation-induced increases in urethral pressure using the same route of administration.

A number of possible explanations for this preferential effect of alfuzosin on urethral responses to phenylephrine can be considered. It is now recognised that multiple subtypes of the α_1-receptor exist (α_{1A}, α_{1B}, α_{1C}, α_{1D} – Lomasney *et al.*, 1991; Faure-Halley *et al.*, 1992) although the functional relevance of these subtypes in terms of contraction of urethral and vascular smooth muscle is far from being fully understood. However, since alfuzosin, like prazosin, does not show marked selectivity for any particular α_1-subtype (D. Graham, C. Pimoule and H. Schoemaker, unpublished observations) this is not likely to be a significant factor. Although the presence of an additional relaxant mechanism in alfuzosin cannot be entirely excluded, testing to date suggests that the compound is a selective α_1-antagonist. In unpublished studies, alfuzosin shows no affinity for histamine, muscarinic, β-adrenoceptor, dopamine, 5-HT_1, 5-HT_2 or benzodiazepine receptors *in vitro* at concentrations up to 10^{-5} M and does not inhibit vasopressor responses to angiotensin, vasopressin or 5-HT in the pithed rat after the high dose of 3 mg kg^{-1}, i.v. Weak affinity for calcium channels (IC_{50} 4.5×10^{-5} M in [^{3}H]-diltiazem binding) and even weaker spasmolytic activity (IC_{50} 9×10^{-4} M in depolarized rabbit aorta) have been identified. However, since these effects occur at concentrations of alfuzosin at least 1000 times greater than those necessary to block α_1-receptors, it is difficult to believe that they could contribute over the dose-range used in the studies performed in the anaesthetized cat. *In vitro*, the α_1-antagonist potency of alfuzosin against phenylephrine is similar in vascular and urinogenital smooth muscle preparations. Thus, the uroselectivity shown by alfuzosin in the cat may therefore be a reflection of an *in vivo* phenomenon. In this context, it is interesting to note that pharmacokinetic studies in the rat have demonstrated that alfuzosin is preferentially distributed in the prostate gland after systemic administration giving a prostate: plasma concentration ratio of $\simeq$3 fold. Therefore, tissue distribution could influence the *in vivo* pharmacological profile of alfuzosin. Clearly, additional studies will be necessary to develop a fuller understanding of these observations including the difference noted between alfuzosin and prazosin. Other relevant examples of *in vivo* differences between alfuzosin and prazosin are reports that alfuzosin causes a lesser effect on the orthostatic reflex in conscious dogs (Cavero *et al.*, 1984b) and is much less potent as an acute antihypertensive agent in spontaneously hypertensive rats (Lefèvre-Borg *et al.*, 1992).

In summary, the data presented demonstrate that alfuzosin is a potent and selective α_1-adrenoceptor antagonist in the lower urinary tract *in vitro* and *in vivo* and has a high affinity for the α_1-receptor of the human prostate. Since these represent the key target tissues in BPH, there is a good pharmacological rationale for expecting alfuzosin to alleviate the dynamic component of urinary obstruction attributable to sympathetic tone in this condition. Clinical studies confirm the efficacy of alfuzosin in BPH and suggest that it is well tolerated with respect to vascular side effects.

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