

Effect of Fiduxosin, an Antagonist Selective for α_{1A} - and α_{1D} -Adrenoceptors, on Intraurethral and Arterial Pressure Responses in Conscious Dogs

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ABSTRACT

Fiduxosin is an α_1 -adrenoceptor antagonist with higher affinity for α_{1A} -adrenoceptors and for α_{1D} -adrenoceptors than for α_{1B} -adrenoceptors. Our hypothesis is that such a compound with higher affinity for subtypes implicated in the control of lower urinary tract function and lower affinity for a subtype implicated in the control of arterial pressure could result in a superior clinical profile for the treatment of lower urinary tract symptoms suggestive of benign prostatic obstruction. The purpose of this study was to evaluate the potency and selectivity of fiduxosin for effects on prostatic intraurethral pressure (IUP) versus mean arterial pressure (MAP) relative to current clinical standards, terazosin and tamsulosin, in conscious dogs. Phenylephrine (PE)-induced increases in IUP and MAP were determined be-

fore and at various time points after an oral dose of each antagonist. Hypotensive potency was also determined. All three antagonists caused dose- and time-dependent blockade of the IUP and MAP pressor effects of PE. The IUP ED₅₀ values of fiduxosin, tamsulosin, and terazosin were 0.24, 0.004, and 0.23 mg/kg p.o., respectively. The corresponding MAP ED₅₀ values were 1.79, 0.006, and 0.09 mg/kg p.o. The rank order of IUP selectivity (ratio) was fiduxosin (7.5-fold), tamsulosin (1.5-fold), and terazosin (0.4 = 2.5-fold MAP-selective). Tamsulosin and terazosin caused dose-dependent hypotension, whereas no change in arterial pressure was seen after fiduxosin. These data, illustrating a superior selectivity profile of fiduxosin, are consistent with our hypothesis.

Lower urinary tract symptoms (LUTS) refer to a constellation of irritative (filling) and obstructive (voiding) symptoms such as frequency, urgency, and slow flow. LUTS is more frequent and severe in older age groups and has a strong negative impact on the quality of life of those affected (Garraway et al., 1991; Girman et al., 1994, 1995; Kirby, 2000). Although LUTS may have a number of underlying etiologies, it is often attributed in older men to benign prostatic enlargement resulting in bladder outlet obstruction. In this subset of cases, it is referred to as symptomatic or clinical benign prostatic hyperplasia (BPH). In the United States, an estimated 5.6 million men suffer, a number that is expected to double by the year 2025 due to increased life expectancy (Chapple, 1999).

α_1 -Adrenoceptor antagonists represent first-line pharmacotherapy for the treatment of LUTS suggestive of benign

prostatic obstruction (BPO). Cloning and pharmacological studies suggest the presence of three distinct subtypes of α_1 -adrenoceptors (α_{1A} , α_{1B} , and α_{1D}) (Hancock, 1996; Zhong and Minneman, 1999). Several studies have shown that the α_{1A} -subtype predominates in the human prostate and that it mediates the contractile effects of norepinephrine in this tissue (Lepor et al., 1993; Forray et al., 1994; Taniguchi et al., 1997). Despite the proven safety and utility of nonselective α_1 -adrenoceptor antagonists such as terazosin and doxazosin in the treatment of clinical BPH, these compounds were initially developed as antihypertensives and can cause arterial pressure related effects that limit dosing. It was hypothesized that α_{1A} -selective antagonists would be more "prostate-selective" thereby improving BPH symptoms with improved tolerability. Indeed, tamsulosin, a compound with enhanced selectivity for α_{1a} - versus α_{1b} -adrenoceptors, rela-

ABBREVIATIONS: LUTS, lower urinary tract symptoms; BPH, benign prostatic hyperplasia; BPO, benign prostatic obstruction; Ro-70-004, 3-(3-{4-[fluoro-2-(2,2,2-trifluoroethoxy)-phenyl]-piperazin-1-yl}-propyl)-5-methyl-1H-pyrimidine-2,4-dione mono hydrochloride monohydrate; IUP, intraurethral pressure; PE, phenylephrine; MAP, mean arterial pressure; fiduxosin (ABT-980), (3-[4-((3aR,9bR)-cis-9-methoxy-1,2,3,3a,4,9b-hexahydro-[1]-benzopyrano[3,4-c]pyrrol-2-yl)butyl]-8-phenyl-pyrazino[2',3':4,5]thieno [3,2-d]pyrimidine-2,4 (1H,3H)-dione hydrochloride); SHR, spontaneously hypertensive rats; A-131701, (3-[2-((3aR,9bR)-cis-6-methoxy-2,3,3a,4,5,9b, hexahydro-[1H]-benz[e]isoindol-2-yl)ethyl]pyrido[3',4':4,5]thieno [3,2-d]pyrimidine-2,4(1H,3H)-dione); REC 15/2739, (N-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4H-1-benzopyran-8-carboxamide); SNAP 5089, 5-[[[3-(4,4-diphenyl-1-piperidinyl)propyl]amino]carbonyl]-1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)-3-pyridinecarboxylic acid methyl ester; REC 15/2627, 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1-oxo-3,3-diphenylpropyl)-piperazine monohydrochloride; RS 17053, N-[2-(2-cyclopropyl methoxy phenoxy)ethyl]5-chloro- α,α -dimethyl-1H-indole-3-ethanamine hydrochloride.

tive to terazosin in vitro (Hancock et al., 2002), is better tolerated clinically due to fewer cardiovascular-related adverse events. However, Ro-70-004, a newer antagonist with even greater subtype selectivity in vitro (≥ 50 -fold for α_{1a} - versus α_{1b} - or α_{1d} -adrenoceptors; Williams et al., 1999) failed to improve LUT symptoms in a clinical trial, although increases in flow rate were observed (Blue et al., 2000). These findings and the long-standing observation that α_1 -adrenoceptor antagonists produce beneficial effects on filling symptoms, even in the absence of obstruction, have stimulated a reevaluation of the previous notion that the clinical efficacy of α_1 -adrenoceptor antagonists is related primarily to relaxation of prostatic smooth muscle alone (Michel et al., 2000). There is an increased appreciation that both prostatic and extraprostatic α_1 -adrenoceptors play a role in lower urinary tract function and may mediate the therapeutic efficacy of α_1 -adrenoceptor antagonists in clinical BPH. Indeed, in addition to the well established role of α_{1A} -adrenoceptors in mediating urethral resistance, α_{1D} -adrenoceptors predominate in detrusor smooth muscle (Malloy et al., 1998) and α_{1D} -adrenoceptor message in that tissue is increased in response to partial outlet obstruction in rats (Hampel et al., 2000). α_{1D} -Adrenoceptors can also be found in areas of the lumbosacral spinal cord involved in mediating voiding reflexes (Smith et al., 1999). Hypotensive potency of α_1 -adrenoceptor antagonists is highly correlated with α_{1b} -affinity and but not to α_{1a} - and to a lesser extent, α_{1d} -affinity (Hancock et al., 2002). An emerging hypothesis is that improved clinical separation between LUT symptom improvement and undesired cardiovascular-related effects may not be related solely to pharmacological selectivity for a single subtype in vitro even though that subtype mediates the contractile effects of α_1 -agonists in LUT smooth muscle. Instead, selectivity for those α_1 -receptors involved in mediating symptoms versus those involved in tonic control of arterial pressure, regardless of subtype, may result in an improved clinical profile. Fiduxosin is a α_{1a} - ($K_i = 0.16$ nM)/ α_{1d} ($K_i = 0.92$ nM)-adrenoceptor antagonist displaying 155-fold selectivity for α_{1a} - versus α_{1b} ($K_i = 25$ nM)-subtypes in radioligand binding studies (Hancock et al., 2000). Its pharmacological profile is suitable for testing the hypothesis that a mixed α_{1A} - and α_{1D} - over α_{1B} -adrenoceptor antagonist would result in a superior therapeutic agent for the treatment of LUTS suggestive of BPO. In this study, we used a conscious dog model to evaluate the effects of fiduxosin on urethral and arterial pressure responses after oral dosing. Using this model, we determined the selectivity of fiduxosin to block prostatic and prostatic urethral α_1 -adrenoceptors over those in the vasculature and compared the results with clinically used agents.¹

Experimental Procedures

Male beagle dogs (>2 years old, 12–15 kg; Marshall Farms, North Rose, NY) were chronically instrumented for the continuous measurement of arterial blood pressure by implanting a telemetry transducer/transmitter (TA11PA-C40; Data Sciences International, St. Paul, MN) into a carotid artery. On test day, dogs were placed in sling restraints and an Abbocath-T i.v. catheter (18-G; Abbott Lab-

oratories, North Chicago, IL) was inserted into a cephalic vein for blood sampling and for the administration of agonist. Prostatic intraurethral pressure (IUP) was measured using a transurethral 7F Swan-Ganz balloon catheter (41224-01; Abbott Laboratories) as previously described (Brune et al., 1995). Dose responses of the intraurethral and arterial pressor effects of 8, 16, and 32 $\mu\text{g}/\text{kg}$ i.v. phenylephrine (PE) were obtained before and at various time points after a single p.o. dose of an antagonist. Fiduxosin was dissolved in a vehicle of 20% ethanol, 30% propylene glycol, and 50% water. Terazosin and tamsulosin were dissolved in water. All antagonists were given by gavage in a volume of 1 ml/kg. PE was dissolved in saline and administered in a volume of 0.1 ml/kg. The increase in IUP or mean arterial pressure (MAP) caused by PE was allowed to return to baseline before the next dose was administered. Dogs were cared for according to National Institutes of Health guidelines on canine care and all experimental protocols described herein were reviewed and approved by the Institutional Animal Care and Use Committee of Abbott Laboratories.

Data Analysis. Data were expressed as percentage of blockade of the baseline pressure responses obtained in the absence of antagonist. Hypotensive effects were expressed as net change from predose MAP and represent the maximum change in MAP seen at any time after dosing. One-way analysis of variance was used to compare the extent of blockade of PE-induced IUP or MAP effects at each time point during the experiment. If statistical significance was indicated, comparisons between groups were performed using Dunnett's multiple range test. ED_{50} values are an estimate of the dose required to cause a maximum inhibition of the IUP or MAP pressor response to PE of 50%. Hypotensive ED_{10} mm Hg values are an estimate of the dose required to produce a maximum decrease in baseline MAP of 10 mm Hg. All ED values were determined by interpolation by using a standard linear regression analysis of doses producing a response just above or below the indicated index value. A paired *t* test was used to compare the maximum blockade values of IUP to MAP after a given antagonist dose. The same test was used to compare duration of effect values of IUP to MAP as well.

Materials. Fiduxosin (ABT-980), terazosin, and tamsulosin were synthesized as hydrochloride salts at Abbott Laboratories. Phenylephrine hydrochloride was purchased from Sigma Chemical (St. Louis, MO).

Results

Baseline control MAP values in all dogs tested ranged from 83 to 110 mm Hg and the overall mean (S.E.M.) was 97 (1.4) mm Hg. Average IUP pressor responses to 8, 16, and 32 $\mu\text{g}/\text{kg}$ i.v. PE in the absence of antagonist were 16 (0.7), 24 (1.1), and 33 (1.3) mm Hg. Corresponding MAP pressor responses to these three PE doses were 27 (1.4), 43 (1.3), and 57 (1.6) mm Hg ($n = 48$). There were no statistical differences in baseline MAP or in the baseline IUP or MAP pressor responses to PE between groups (one-way analysis of variance; data not shown). When normalized to the corresponding control agonist response, any dose of an α_1 -adrenoceptor antagonist produced similar attenuation of all three PE doses (data not shown). Therefore, although for clarity only data obtained after administration of the 32- $\mu\text{g}/\text{kg}$ i.v. dose is presented herein, the results for all antagonists at this PE dose were representative of those obtained after the 8 and 16 $\mu\text{g}/\text{kg}$ i.v. The relative potencies and selectivities of these antagonists were not dependent on the agonist dose (data not shown).

Some attenuation of the MAP and IUP pressor responses to PE was seen over time in the absence of antagonists in the vehicle only group (Figs. 1–3). The reasons for this are unclear but could be associated with handling conscious ani-

¹ In this article, nomenclature used to differentiate among the subtypes of α_1 -adrenoceptors uses uppercase subscripted letters to describe tissue-sourced receptors and lowercase subscripted letters to define cloned receptors (Bylund et al., 1994).

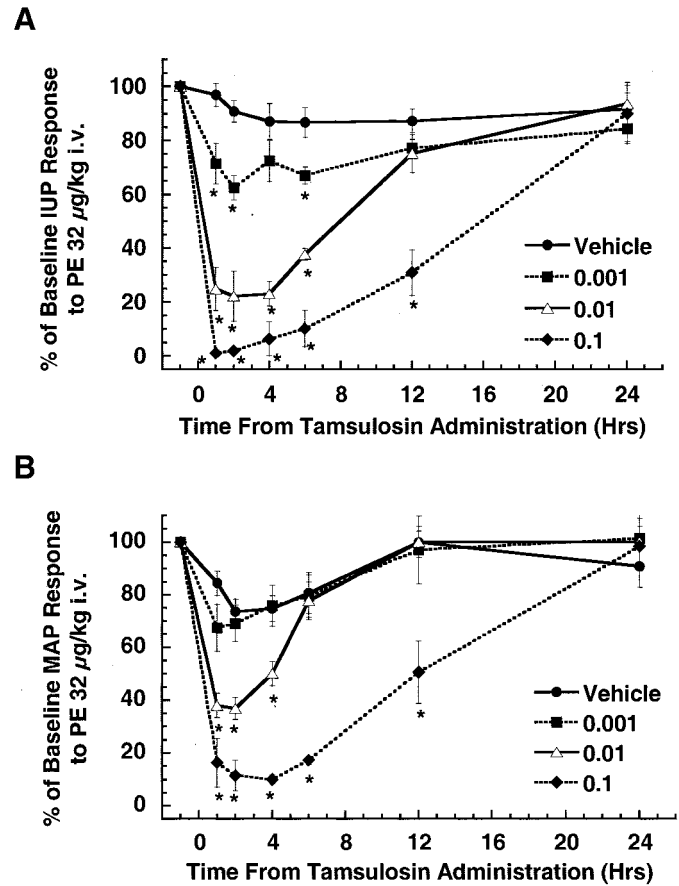
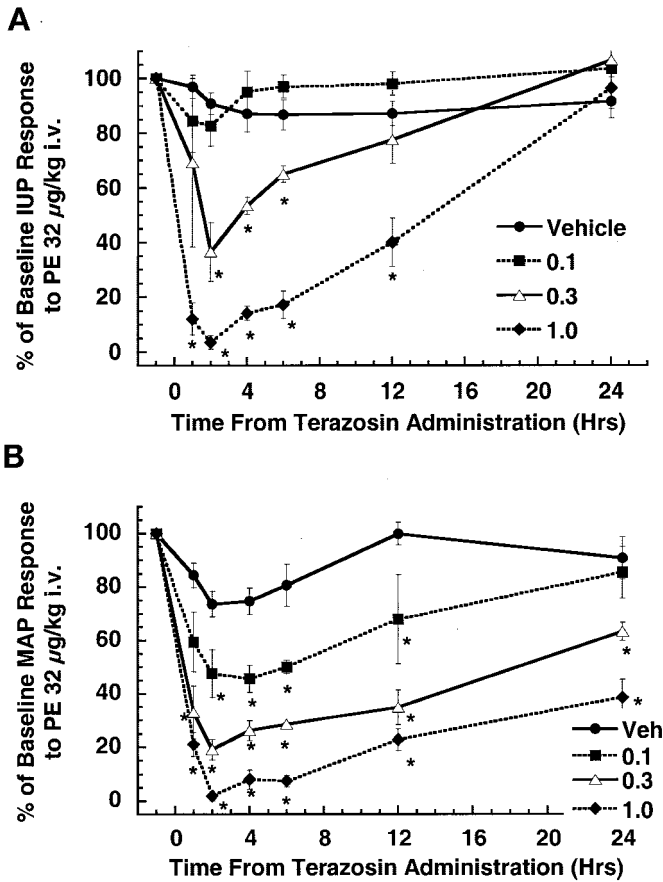


Fig. 1. Time course effects of terazosin to block PE-induced increases in IUP (A) and block PE-induced increases in MAP (B) measured simultaneously in conscious dogs. Doses are mg/kg p.o. as indicated in the inset. Data represent mean \pm S.E.M., $n = 4$ to 5/dose. * $p \leq 0.05$ compared with vehicle.

Fig. 2. Time course effects of tamsulosin to block PE-induced increases in IUP (A) and block PE-induced increases in MAP (B) measured simultaneously in conscious dogs. Doses are mg/kg p.o. as indicated in the inset. Data represent mean \pm S.E.M., $n = 4$ to 5/dose. * $p \leq 0.05$ compared with vehicle.

mals as previously discussed (Brune et al., 1996) or receptor desensitization with repeated agonist administration. Regardless, these effects were small relative to those evoked by the antagonists.

Figures 1 to 3 illustrate the time course of antagonist inhibition of the IUP and MAP pressor effects of PE. As these figures indicate, all three antagonists dose dependently inhibited the IUP and MAP pressor responses to i.v. PE. There were differences between compounds in the magnitude of blockade of each simultaneously measured parameter. Terazosin, for example, always blocked the MAP pressor response to an equal or greater extent than IUP (Fig. 1, A and B). The maximum percentage of inhibition (E_{max}) of the MAP pressor response after terazosin at 0.1, 0.3, and 1.0 mg/kg p.o. was 60, 82, and 98, whereas the corresponding IUP E_{max} values were 23, 67, and 97. Selectivity at each dose as measured by IUP E_{max} - MAP E_{max} was -37, -15, and -1, indicating that terazosin is "MAP-selective" at submaximally effective doses (Fig. 4A). The time course effects of tamsulosin in the model are included in Fig. 2, A and B. The same selectivity analysis applied to the tamsulosin IUP inhibition E_{max} values (40, 82, and 100%) minus corresponding MAP E_{max} values (38, 66, and 90%) yielded differences of 2, 16, and 10% at 0.001, 0.01, and 0.1 mg/kg p.o., respectively (Fig. 4B). Although the absolute IUP selectivity of tamsulosin is modest, these data indicate some degree of IUP selectivity of tamsulosin relative to terazosin.

Fiduxosin (0.1, 0.3, 1.0, and 3.0 mg/kg p.o.) always blocked IUP responses to a greater extent than MAP responses (Fig. 3, A and B). IUP inhibition E_{max} values at each respective increasing dose (34, 60, 75, and 91) were greater than corresponding MAP E_{max} values (27, 35, 36, and 71) by 7, 24, 39, and 20% (Fig. 4C). These differences exceeded those obtained after either terazosin or tamsulosin as shown above.

In Fig. 6A, the same maximum inhibition data set was used to estimate a dose of terazosin or tamsulosin that produces IUP blockade equal to that seen with fiduxosin at 1.0 mg/kg p.o. (75%). Then the degree of MAP blockade at these equieffective IUP doses was estimated. The doses of terazosin and tamsulosin estimated to block IUP responses by 75% were 0.46 and 0.0085 mg/kg p.o. Corresponding MAP blockade values were much less for fiduxosin (38%) than either terazosin (86%) or tamsulosin (61%). These data indicate a uroselectivity rank order of fiduxosin > tamsulosin > terazosin.

We also examined the duration of blockade of each parameter as an indication of selectivity. Figure 5, A to C, illustrate the length of time after each compound where the duration of IUP or MAP blockade exceeded 50%. As indicated in Fig. 6A, terazosin blocked MAP responses several hours longer than IUP responses at all doses. Only after the 0.01-mg/kg dose of tamsulosin, where IUP selectivity was greatest by the comparison of peak responses described above, did IUP blockade

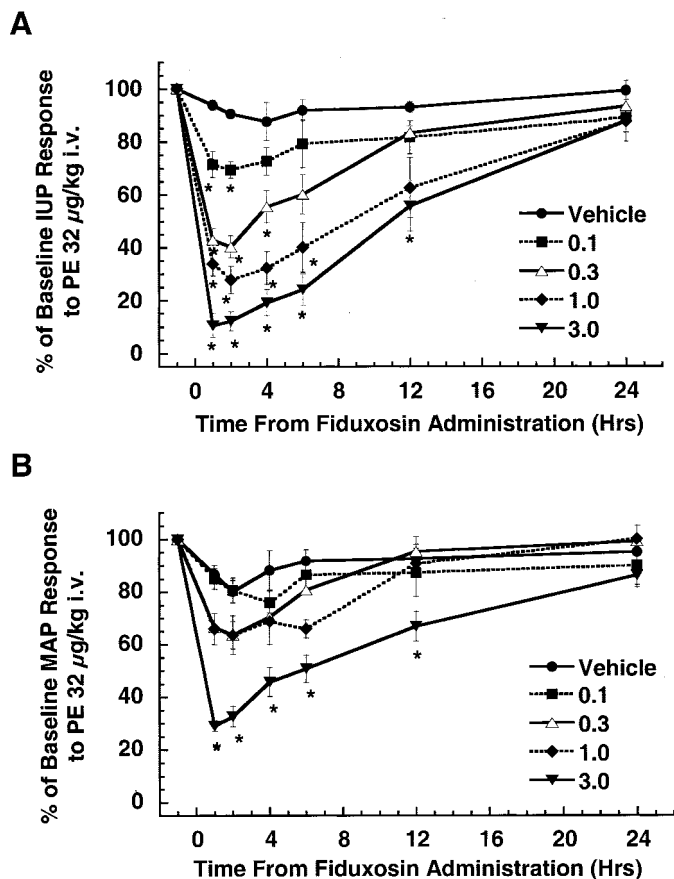


Fig. 3. Time course effects of fiduxosin to block PE-induced increases in IUP (A) and block PE-induced increases in MAP (B) measured simultaneously in conscious dogs. Doses are mg/kg p.o. as indicated in the inset. Data represent mean \pm S.E.M., $n = 4$ to 5/dose. * $p \leq 0.05$ compared with vehicle.

duration significantly exceed that of MAP (Fig. 5B). That modest dose and time-specific selectivity is also illustrated by examining the dose-response data in Fig. 2, A and B. At 6 h after 0.01 mg/kg tamsulosin, the MAP response had returned to baseline but the IUP response was still significantly blocked (62%). In contrast, fiduxosin blocked IUP responses significantly longer than MAP responses at all doses. Consistent with the analysis of maximal blockade above, the duration difference was greatest after 1 mg/kg (Fig. 5C).

A similar analysis to that described above for E_{\max} data was used to estimate the dose of terazosin or tamsulosin that would cause an IUP blockade greater than 50% for a duration equal to that achieved by fiduxosin at 1.0 mg/kg p.o. (9 h). MAP blockade duration of an effect greater than 50% at these equieffective IUP doses was then estimated. Doses of terazosin and tamsulosin estimated to block IUP responses greater than 50% for 9 h were 0.7 and 0.022 mg/kg p.o., respectively. Corresponding MAP blockade duration values were less for fiduxosin (0 h) than either terazosin (21 h) or tamsulosin (5 h; Fig. 6B). These data also indicate a uroselectivity rank order of fiduxosin > tamsulosin > terazosin.

Hypotensive effects of these antagonists were also monitored in these dogs and the dose-response data are summarized in Fig. 7. Maximum changes after the two vehicles used in this study (ethanol, propylene glycol, water and water alone) were +4 and -2.5%, respectively (data not shown).

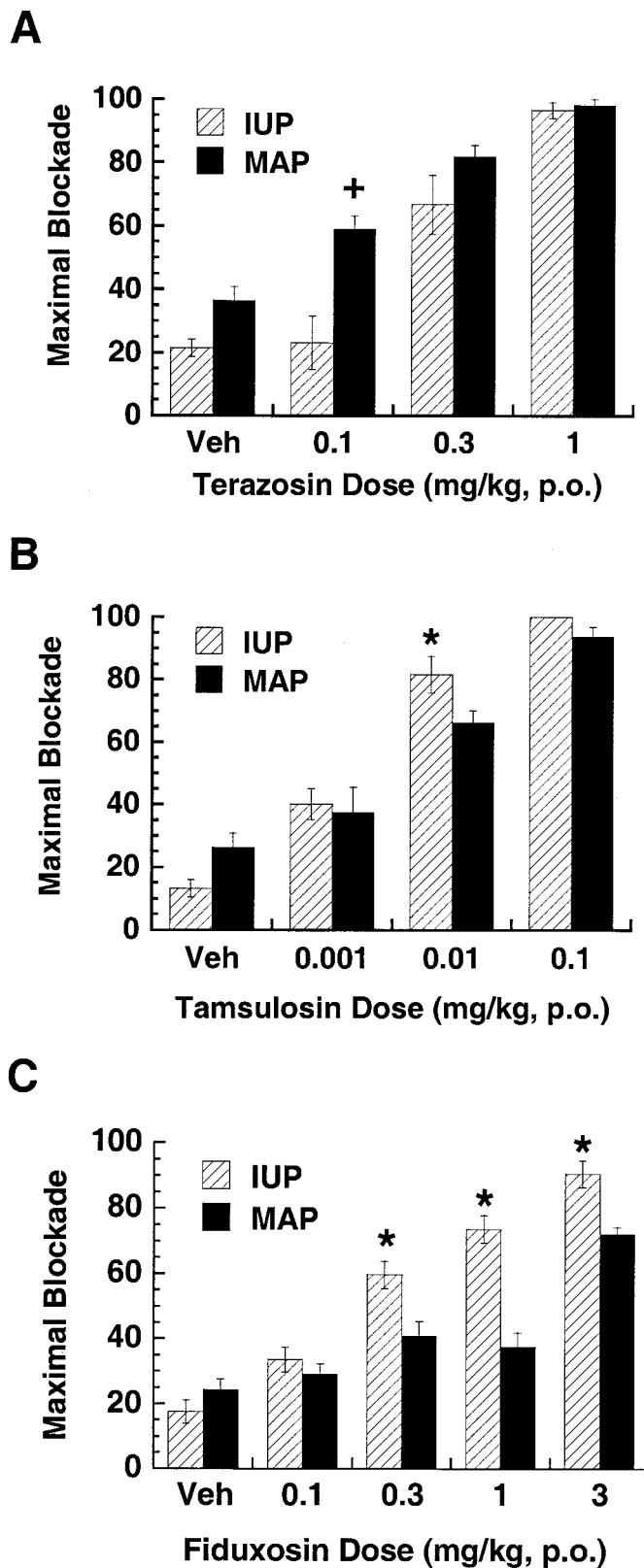


Fig. 4. Maximal blockade of PE-induced increases in IUP (▨) or MAP (■) seen after oral dosing with terazosin (A), tamsulosin (B), or fiduxosin (C). Data represent mean \pm S.E.M. of four to five dogs per dose. Data analyzed using paired t test. + $p \leq 0.05$ MAP blockade maximums exceeded IUP blockade maximums; * $p \leq 0.05$ IUP blockade maximums exceeded MAP blockade maximums.

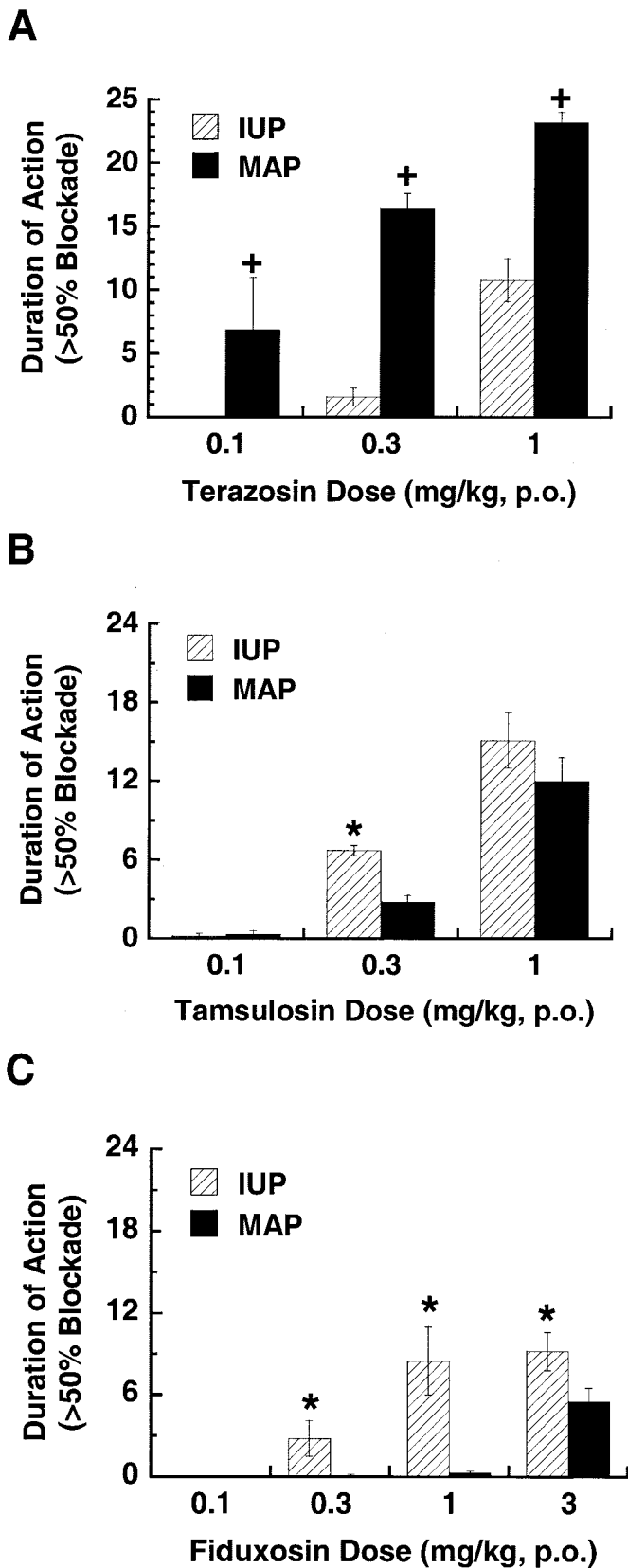


Fig. 5. Duration of antagonist-induced IUP (▨) or MAP (■) pressor response blockade exceeding 50% of the predose baseline. Data represent mean ± S.E.M. of four to five dogs per dose. Data analyzed using paired *t* test. ⁺*p* ≤ 0.05 MAP blockade duration exceeded IUP blockade duration; **p* ≤ 0.05 IUP blockade duration exceeded MAP blockade duration.

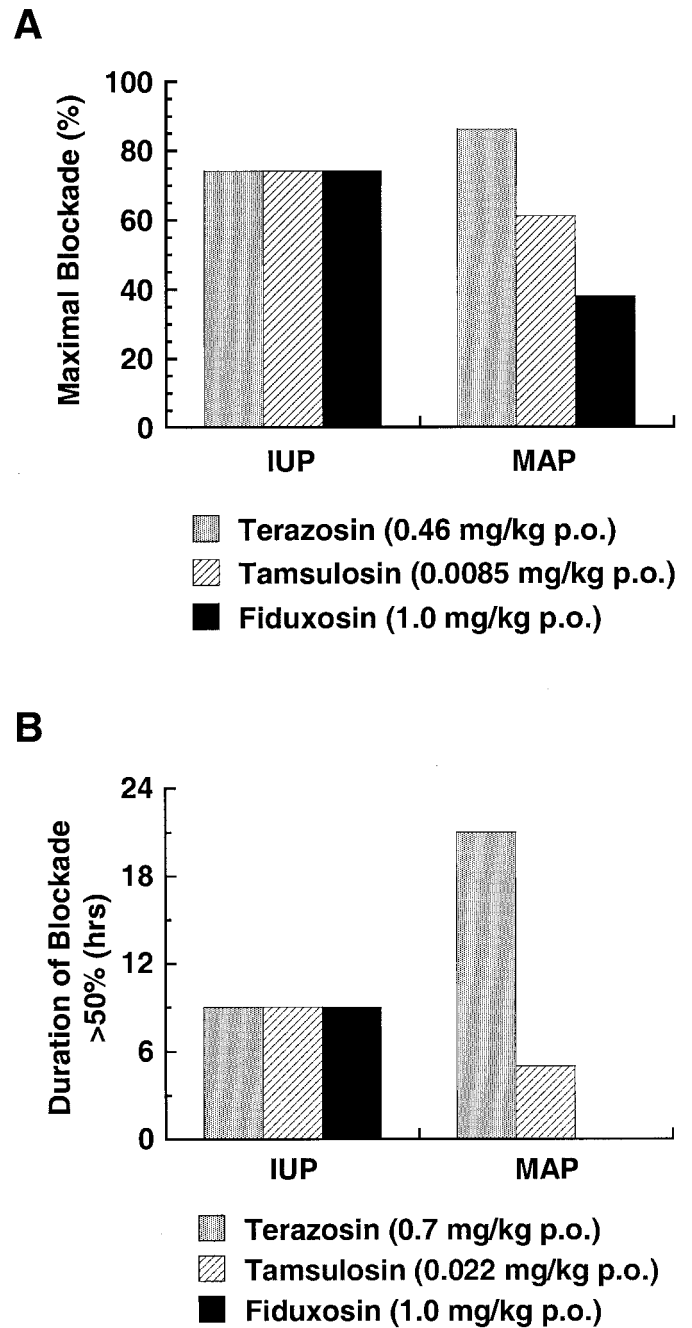


Fig. 6. A, estimates of the maximal blockade of PE-induced MAP pressor effects at doses of each antagonist that produce an equivalent IUP blockade maximal effect of 75%. “Uroselectivity” is, therefore, inversely proportional to the extent of MAP blockade at these IUP equieffective doses. B, doses of each antagonist were estimated that produce an equivalent duration of IUP effect where pressor effects were blocked >50% of baseline. Because IUP effect durations are equivalent, uroselectivity is inversely proportional to MAP effect duration. As indicated in Fig. 3, MAP blockade after fiduxosin was always less than 50% at 1 mg/kg p.o., therefore, the MAP duration bar heights are zero. These analyses suggest the same antagonist rank order of uroselectivity: fiduxosin > tamsulosin > terazosin.

Also, our practical experience suggests changes in MAP of ±5 mm Hg were within the noise range of this assay, and therefore were not considered biologically significant. As Fig. 7 indicates, terazosin and tamsulosin caused dose-dependent hypotension, whereas fiduxosin did not affect baseline arterial pressure at any dose tested.

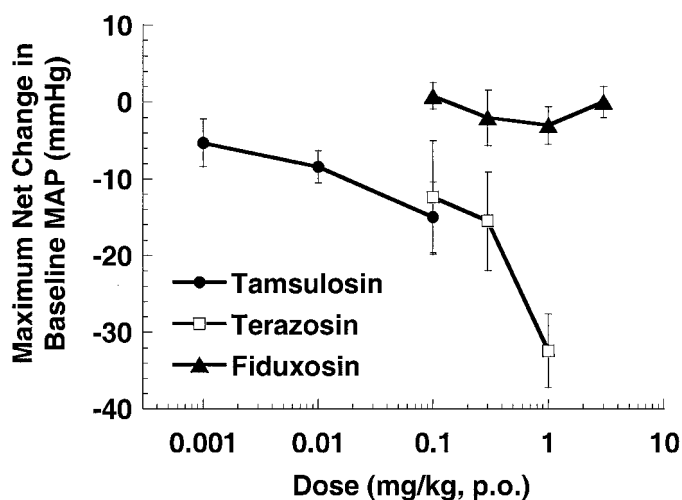


Fig. 7. Dose-response effects of terazosin, tamsulosin, and fiduxosin to decrease resting MAP in conscious dogs. A net change greater than 5 mm Hg was considered to be above the noise level associated with this assay. Terazosin and tamsulosin caused dose-dependent hypotension. Fiduxosin did not cause hypotension at any dose tested.

To summarize the results, the doses of each antagonist required to produce the following effects were calculated: 1) maximum 50% inhibition of IUP pressor effect, 2) maximum 50% inhibition of MAP pressor effect, and 3) 10 mm Hg decrease in baseline MAP. These potency indices and resulting selectivity ratios are summarized in Table 1.

Discussion

The results of the present study illustrate clear differences in the α_1 -adrenoceptor antagonist potency and pharmacological selectivity of these compounds in vivo. Tamsulosin was the most potent compound and was somewhat IUP-selective relative to terazosin, a compound that was consistently MAP-selective in the assay. Fiduxosin was clearly the most IUP-selective compound by using either a comparison with MAP E_{max} values or blockade effect duration. The selectivity comparison in Fig. 5 where MAP blockade was less at equieffective doses of the antagonists that produce 75% inhibition may be particularly relevant. In a previous report modeling the relationship between the plasma levels of terazosin and pharmacodynamic effects in this model (Witte et al., 1997), the estimated plasma concentration of terazosin to produce 75% blockade was 100 ng/ml, which is equal to peak plasma levels achieved clinically with 5 mg of terazosin (Taguchi et al., 1998).

Fiduxosin also demonstrated superior IUP selectivity over hypotensive effects, which is of potential clinical importance. Unfortunately, the true selectivity of fiduxosin in this regard may be underestimated due to a lack of hypotensive effects in these normotensive animals. Indeed, the hypotensive effects of α_1 -adrenoceptor antagonists used clinically to treat LUTS suggestive of BPO are minimal at effective doses in normotensives but these agents do lower arterial pressure in hypertensives. The lack of hypotensive potency of fiduxosin relative to terazosin and tamsulosin seen herein was confirmed in conscious SHR, a model highly sensitive to the depressor effects of α_1 -antagonists (Hancock et al., 2002). These data indicating a similar rank order of antagonist potency to produce hypotension in both normotensive dogs

and hypertensive rats are consistent with the notion that similar subtype populations mediate the hypotensive response in both species. The increased magnitude of antagonist responses in SHR and hypertensive patients could be explained by the known role of enhanced sympathetic outflow in hypertension (Tuck, 1986).

These data taken together suggest a compound such as fiduxosin may have an improved cardiovascular liability profile. The enhanced relative selectivity shown herein of tamsulosin compared with terazosin is consistent with the selectivity rank order reported in other models (Hancock et al., 2002; Witte et al., 2002) and in clinical studies (Djavan and Marberger, 1999; Schafers et al., 1999). The in vitro and in vivo profile of fiduxosin is similar to that of another α_{1A} - and α_{1B} -selective antagonist, A-131701 (Hancock et al., 1998a,b).

In this study, the uroselectivity of these α_1 -adrenoceptor antagonists versus hypotensive effects exceeded their uroselectivity versus α_1 -adrenoceptor agonist-induced increase in MAP (Table 1). One theoretical explanation is that hypotension and blockade of agonist-induced pressor effects are mediated by distinct receptor populations. For example, if the pressor responses to an exogenous intravenous agonist and the physiological release of endogenous agonist from sympathetic nerves are mediated by different subtypes or by receptors in different anatomic locations, the pharmacology of the chosen agonist could impact the findings, including perhaps implying a role for a particular receptor subtype not relevant to normal physiology. For example, although highly α_{1A} -selective antagonists lack potency to decrease MAP (Blue et al., 1997), highly α_{1A} -selective agonists potentially increase it (Knepper et al., 1995; Taniguchi et al., 1996). A unifying hypothesis to explain these observations is that there may be α_{1A} -adrenoceptors located extrajunctionally that are not involved in the tonic sympathetic noradrenergic control of arterial pressure but that mediate an exogenous α_{1A} -adrenoceptor agonist-induced increase in pressure. Therefore, the pressor response to the exogenous administration of an α_{1A} -agonist such as PE or its functional blockade by an α_{1A} -antagonist is not necessarily indicative of a role for these receptors in the tonic sympathetic control of resting arterial pressure. If this extrajunctional hypothesis is accurate, antagonist affinity for α_{1A} -adrenoceptors that mediate IUP effects would simultaneously result in blockade of α_{1A} -adrenoceptor agonist-induced MAP pressor effects, thereby having a self-limiting effect on an estimate of selectivity. A study that compares the rank order of potency of subtype selective antagonists to block MAP pressor effects caused by stimulation of lumbar sympathetic nerves with their ability to block agonist-induced increases could further elucidate the functional role of α_1 -adrenoceptor subtypes in the cardiovascular system.

In contrast to arterial pressure where multiple α_1 -adrenoceptor subtypes are involved in its regulation, both agonist-induced increases and sympathetic nerve-mediated increases in urethral pressure appear mediated the same (α_{1A}) subtype. For example, similar doses of REC 15/2739 (α_{1A} -selective), tamsulosin, or terazosin blocked norepinephrine-induced and hypogastric nerve-induced increases in urethral pressure to a similar extent (Leonardi et al., 1997). Furthermore, the rank order of antagonist potency was the same as their affinity for the α_{1A} - but not α_{1B} - and α_{1D} -subtypes. It would be expected that the selectivity profile of a mixed

TABLE 1

Summary of the effects of terazosin, tamsulosin, and fiduxosin on simultaneously measured IUP or arterial pressure parameters in conscious dogs. The effective doses of each antagonist required to produce the following effects were calculated: maximum 50% inhibition of IUP pressor effect (1), maximum 50% inhibition of MAP pressor effect and (2), 10 mm Hg decrease in baseline MAP (3). Uroselectivity ratios are summarized in the last two columns. Using the first two indices, the IUP selectivity of fiduxosin (7.5-fold) was superior to that of tamsulosin (1.5-fold) or terazosin (ratio = 0.4- or 2.5-fold MAP-selective). IUP selectivity as defined by IUP versus hypotensive effects yields the same rank order: fiduxosin (>12.5-fold) > tamsulosin (7.5-fold) > terazosin (0.7-fold). The actual uroselectivity of fiduxosin may be underestimated as the absence of hypotension after fiduxosin precludes an absolute estimate.

| | IUP ED ₅₀ (1) | MAP ED ₅₀ (2) | Hypotensive ED ₁₀ mm Hg (3) | Selectivity Ratio | |
|------------|--------------------------|--------------------------|--|-------------------|---------|
| | | | | (2)/(1) | (3)/(1) |
| Fiduxosin | 0.24 (0.08) | 1.79 (0.47) | >3.0 | 7.5 | >12.5 |
| Tamsulosin | 0.004 (0.003) | 0.006 (0.005) | 0.03 (0.13) | 1.5 | 7.5 |
| Terazosin | 0.23 (0.09) | 0.09 (0.06) | 0.16 (0.11) | 0.4 | 0.7 |

subtype agonist such as PE would not be a confounding factor in the case where a single subtype mediates the functional responses of the agonist.

For most α_1 -adrenoceptor antagonists, including fiduxosin, there is a strong positive correlation between affinity for the LUT predominant (α_{1A}) subtype and functional antagonist potency on LUT tissue in vitro and in vivo (Hancock, 1996; Hancock et al., 2002). However, some compounds have been described that have much weaker functional potency in prostatic tissue in vitro than would be predicted by their affinities for the recombinant α_{1A} -subtype such as SNAP 5089, REC 15/2627 (Leonardi et al., 1997), and RS 17053 (Ford et al., 1996). This has led to the hypothesis that contraction of prostatic (and urethral) tissue is mediated at least in part by an atypical α_1 -adrenoceptor subtype, possibly the putative α_{1L} -subtype (low affinity for prazosin) initially proposed by Flavahan and Vanhoutte (1986) and extended by Murumatsu et al. (1990, 1995) to explain the differences in the affinity constants for prazosin in different vascular tissue assays in vitro. Current data support the notion that the α_{1L} -“subtype” may not be a distinct molecular entity but rather as a different “affinity state” of the α_{1A} -adrenoceptor gene product. The fiduxosin in vitro data (Hancock et al., 2002) suggests that, unlike the outlier compounds described above and like most other α_1 -adrenoceptor antagonists, the affinity of fiduxosin for the α_{1A} -adrenoceptor is independent of its affinity state.

In a previous article on the effects of tamsulosin and terazosin in this model (Brune et al., 1996), the results were reported using PE at 16 $\mu\text{g}/\text{kg}$ i.v., whereas 32 $\mu\text{g}/\text{kg}$ i.v. is used herein. We routinely administered three PE doses in the protocol not knowing a priori where these three doses might lie on the dose-response curve in a particular dog. For example, if PE 32 $\mu\text{g}/\text{kg}$ i.v. was a supramaximal IUP dose on a given day, an antagonist might cause less IUP blockade and therefore look less selective. Comparing the effects of multiple agonist doses helps evaluate compounds as to their ability to shift a dose response to the right instead of inhibition of effects at only a single dose. In hindsight, antagonist blockade of all PE doses was similar in different dogs on different days, indicating that these doses all lie on the “steep” portion of the dose-response curve and that compound potency and selectivity was not affected by such issues.

In this study, we demonstrated the utility of a conscious dog model to demonstrate selective functional α_1 -adrenoceptor antagonism in the lower urinary tract (urethra and prostate) compared with the vascular system. This model appears relevant because 1) the α_{1A} -adrenoceptor subtype is predominant in the LUT of both dog and human, 2) the pharmacology of dog lower urinary tract α_1 -adrenoceptors is similar to that of human (Lepor et al., 1992, 1994), and 3) androgen-

dependent macroscopic BPH occurs in both dog and human with increasing age (Johnston et al., 2000). Although the extent of similarity between dog and human with respect to distribution of α_1 -adrenoceptor subtypes in the vasculature remains to be fully elucidated, the clinical observation that tamsulosin is better tolerated than terazosin due to less of an effect on arterial pressure was predicted by this study and by a similar PE challenge protocol in humans (Schafers et al., 1999). Conscious animals also enable the assessment of pharmacodynamic effects after oral dosing without the confounding effects of anesthesia and to relate those effects to pharmacokinetic parameters. However, the relationship between IUP as measured in this model, bladder outlet resistance, and ultimately, clinical symptom improvement is complex and not fully understood. Dynamic bladder outlet resistance is not routinely measured because it requires the difficult simultaneous measurement of pressure and flow (resistance = pressure/flow). Instead, IUP is used as a surrogate measure that directly reflects changes in resistance assuming flow is constant. The IUP measurement mostly reflects changes in the tone of smooth muscle surrounding the balloon (prostate and prostatic urethra) and not bladder or reflex effects because it is measured in the absence of a bladder contraction. Most of the agonist-induced increase in IUP can be attributed to effects on the prostate because agonist-induced increases were 60 to 80% smaller in the corresponding location in female dogs or in males when the balloon was placed just proximal or just distal to the prostatic urethra (Brune et al., 1995).

The relationship between the prostate, obstruction, and symptoms is also complex. LUT symptoms are classified as either obstructive such as weak stream and incomplete emptying or irritative such as frequency and urgency. Although in most patients surgical resection of the prostate relieves obstruction and improves both types of symptoms, about 30% of surgical patients do not show improvement in irritative symptoms. These data suggest that the amelioration of obstruction and the corresponding decrease in urethral resistance are not the only factors that determine symptom relief. This ultimate goal of therapy, symptom improvement, cannot be modeled easily, if at all, in laboratory animals. The relevance of α_1 -adrenoceptor antagonist effects on IUP to therapeutic efficacy in LUTS suggestive of BPO remains to be fully elucidated.

A balanced antagonist profile across α_1 -adrenoceptor subtypes implicated in the control of bladder function and outlet resistance (α_{1A} and α_{1D}) without cardiovascular effects at lower urinary tract-effective doses may result in a superior therapeutic agent. Fiduxosin demonstrates this balanced antagonist profile in radioligand binding and in vitro functional

studies. In this current study, in an *in vivo* model that predicted the improved clinical uroselectivity of tamsulosin over terazosin, fiduxosin demonstrated selectivity superior to these agents. The improved uroselectivity of fiduxosin demonstrated herein, particularly versus hypotensive effects, suggest an α_1 -adrenoceptor antagonist with this profile may be of therapeutic potential in the treatment of LUTS suggestive of BPO.

References

- Blue DR, Ford APDW, Morgans DJ, Williams TJ, Zhu Q-M, and Clarke DE (1997) The conscious "reflex-compromised" rat: a model for evaluating the hypotensive potencies of the α_1 -adrenoceptor antagonists prazosin, tamsulosin and Ro 70-004. *Br J Pharmacol* **120**:107P.
- Blue DR Jr, Grino PB, Jung DT, Harbison MP, and Ford APDW (2000) Evaluation of Ro 70-0004, a selective α_{1A} -adrenoceptor antagonist, in men with benign prostatic hyperplasia (BPH) (Abstract). *Fifth International Consultation on BPH*; 2000 June 25–28; Paris, France; 550 p, World Health Organization (WHO), Geneva, Switzerland.
- Brune ME, Buckner SA, Polakowski J, Kerwin JF Jr, and Hancock AA (1995) Pharmacological antagonism of α -adrenergic agonist induced increases in canine intraurethral pressure *in vivo*. *Drug Dev Res* **34**:267–275.
- Brune ME, Katwala SP, Milicic I, Buckner SA, Ireland LM, Kerwin JF Jr, and Hancock AA (1996) Effects of selective and nonselective α_1 -adrenoceptor antagonists on intraurethral and arterial pressures in intact conscious dogs. *Pharmacology* **53**:356–368.
- Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR Jr, and Trendelenburg U (1994) IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol Rev* **46**:121–136.
- Chapple CR (1999) Introduction and concluding remarks. *Eur Urol* **36** (Suppl 3):1–6.
- Djavan B and Marberger M (1999) A meta-analysis on the efficacy and tolerability of α_1 -adrenoceptor antagonists in patients with lower urinary tract symptoms suggestive of benign prostatic obstruction. *Eur Urol* **36**:1–13.
- Flavahan NA and Vanhoutte PM (1986) α -Adrenoceptor subclassification in vascular smooth muscle. *Trends Pharmacol Sci* **7**:347–349.
- Ford AP, Arredondo NF, Blue DR Jr, Bonhaus DW, Jasper J, Kava MS, Lesnick J, Pfister JR, Shieh IA, Vimont RL, et al. (1996) RS-17053 (*N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro- α , α -dimethyl-1*H*-indole-3-ethanamine hydrochloride), a selective α_1A -adrenoceptor antagonist, displays low affinity for functional α_1 -adrenoceptors in human prostate: implications for adrenoceptor classification. *Mol Pharmacol* **49**:209–215.
- Furray C, Bard JA, Wetzel JM, Chiu G, Shapiro E, Tang R, Lepor H, Hartig PR, Weinschank RL, Brancheck TR, et al. (1994) The α_1 -adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned α_{1C} subtype. *Mol Pharmacol* **45**:703–708.
- Garraway WM, Collins GN, and Lee RJ (1991) High prevalence of benign prostatic hypertrophy in the community. *Lancet* **338**:469–471.
- Girman CJ, Epstein RS, Jacobsen SJ, Guess HA, Panser LA, Oesterling JE, and Lieber MM (1994) Natural history of prostatism: impact of urinary symptoms on quality of life in 2115 randomly selected community men. *Urology* **44**:825–831.
- Girman CJ, Jacobsen SJ, Guess HA, Oesterling JE, Chute CG, Panser LA, and Lieber MM (1995) Natural history of prostatism: relationship among symptoms, prostate volume and peak urinary flow rate. *J Urol* **153**:1510–1515.
- Hampel C, Dolber PC, Savic SL, Schwinn DA, Thuroff JW, and Thor KB (2000) Changes in α_1 adrenergic receptor (AB) subtype gene expression during bladder outlet obstruction of rats. *J Urol* **164** (Suppl 4):228.
- Hancock AA (1996) α_1 -Adrenoceptor subtypes: a synopsis of their pharmacology and molecular pharmacology. *Drug Dev Res* **39**:54–107.
- Hancock AA, Brune ME, Witte DG, Marsh KC, Katwala S, Milicic I, Ireland LM, Crowell D, Meyer MD, and Kerwin JF Jr (1998a) Actions of A-131701, a novel, selective antagonist for α_1A - compared to α_1B -adrenoceptors on intraurethral and blood pressure responses in conscious dogs and a pharmacodynamic assessment of *in vivo* prostatic selectivity. *J Pharmacol Exp Ther* **285**:628–642.
- Hancock AA, Buckner SA, Brune ME, Katwala S, Milicic I, Ireland LM, Kang CH, Morse PA, Knepper SM, Meyer MD, et al. (1998b) Pharmacological characterization of A-131701, a novel, α_1 -adrenoceptor antagonist selective for α_1A - and α_1D - compared to α_1B -adrenoceptors. *Drug Dev Res* **44**:140–162.
- Hancock AA, Meyer MD, Brune ME, Buckner S, Esbenshade T, Drizin I, Sullivan JP, Williams M, and Kerwin JF Jr (2000) Fiduxosin—An α_{1AD} receptor antagonist with enhanced *in vivo* uroselectivity relative to terazosin and tamsulosin. *J Urol* **163** (Suppl 4): 310.
- Hancock AA, Buckner SA, Brune ME, Esbenshade TA, Ireland LM, Katwala S, Milicic I, Meyer MD, Kerwin JF Jr, and Williams M (2002) Preclinical pharmacology of fiduxosin (ABT-980), a novel, α_1 -adrenoceptor antagonist with uroselective properties. *J Pharmacol Exp Ther* **300**:478–486.
- Johnston SD, Kamolpatana K, Root-Kustritz MV, and Johnston GR (2000) Prostatic disorders in the dog. *Anim Reprod Sci* **60–61**:405–415.
- Kirby RS (2000) The natural history of benign prostatic hyperplasia: what have we learned in the last decade? *Urology* **56**:3–6.
- Knepper SM, Buckner SA, Brune ME, DeBernardis JF, Meyer MD, and Hancock AA (1995) A-61603, a potent α_1 -adrenergic receptor subtype agonist, selective for the α_{1A} subtype. *J Pharmacol Exp Ther* **274**:97–103.
- Leonardi A, Hieble JP, Guarneri L, Naselsky DP, Poggesi E, Sironi G, Sulpizio AC, and Testa R (1997) Pharmacological characterization of the uroselective α_1A - antagonist Rec 15/2739 (SB 216469): role of the α_1L adrenoceptor in tissue selectivity, part I. *J Pharmacol Exp Ther* **281**:1272–1283.
- Lepor H, Tang R, Mertzyk S, Hartanto V, and Shapiro E (1992) Binding and functional properties of α_1 -adrenoceptors and the area density of smooth muscle in the canine prostate. *J Urol* **148**:1310–1313.
- Lepor H, Tang R, and Shapiro E (1993) The α_1 -adrenoceptor subtype mediating the tension of human prostatic smooth muscle. *Prostate* **22**:301–307.
- Lepor H, Zhang W, Kobayashi S, Tang R, Wang B, and Shapiro E (1994) A comparison of the binding and functional properties of α_1 -adrenoceptors and area density of smooth muscle in the human, canine and rat prostates. *J Pharmacol Exp Ther* **270**:722–727.
- Malloy BJ, Price DT, Price RR, Bienstock AM, Dole MK, Funk BL, Rudner XL, Richardson CD, Donatucci CF, and Schwinn DA (1998) α_1A -adrenergic receptor subtypes in human detrusor. *J Urol* **160**:937–943.
- Michel MC, Schafers RF, and Goepel M (2000) α_1 -Blockers and lower urinary tract function: more than smooth muscle relaxation? *BJU Int* **86** (Suppl 2):23–30.
- Muramatsu I, Ohmura T, Hashimoto S, and Oshita M (1995) Functional subclassification of vascular α_1 -adrenoceptors. *Pharmacol Comm* **6**:23–28.
- Muramatsu I, Ohmura T, Kigoshi S, Hashimoto S, and Oshita M (1990) Pharmacological subclassification of α_1 -adrenoceptors in vascular smooth muscle. *Br J Pharmacol* **99**:197–201.
- Schafers RF, Fokuhl B, Wasmuth A, Schumacher H, Taguchi K, de Mey C, Philipp T, and Michel MC (1999) Differential vascular α_1A -adrenoceptor antagonism by tamsulosin and terazosin. *Br J Clin Pharmacol* **47**:67–74.
- Smith MS, Schambra UB, Wilson KH, Page SO, and Schwinn DA (1999) α_1A -adrenergic receptors in human spinal cord: specific localized expression of mRNA encoding α_1A -adrenergic receptor subtypes at four distinct levels. *Brain Res Mol Brain Res* **63**:254–261.
- Taguchi K, Schäfers RF, and Michel MC (1998) Radioreceptor assay analysis of tamsulosin and terazosin pharmacokinetics. *Br J Clin Pharmacol* **45**:49–55.
- Taniguchi T, Hamada K, Ogasawara T, Ukai Y, Yoshikuni Y, and Kimura K (1996) NS-49, an α_{1A} -adrenoceptor agonist, selectively increases urethral pressure in dogs. *Eur J Pharmacol* **318**:117–122.
- Taniguchi N, Ukai Y, Tanaka T, Yano J, Kimura K, Moriyama N, and Kawabe K (1997) Identification of α_1 -adrenoceptor subtypes in the human prostatic urethra. *Naunyn-Schmiedeberg's Arch Pharmacol* **355**:412–416.
- Tuck ML (1986) The sympathetic nervous system in essential hypertension. *Am Heart J* **112**:877–886.
- Williams TJ, Blue DR, Daniels DV, Davis B, Elworthy T, Gever JR, Kava MS, Morgans D, Padilla F, Tassa S, et al. (1999) *In vitro* α_1 -adrenoceptor pharmacology of Ro 70-0004 and RS-100329, novel α_1A -adrenoceptor selective antagonists. *Br J Pharmacol* **127**:252–258.
- Witte DG, Brune ME, Katwala SP, Milicic I, Kerwin JF Jr, and Hancock AA (1997) Relationships between pharmacokinetics and blockade of agonist-induced prostatic intraurethral pressure and mean arterial pressure in the conscious dog after single and repeated daily oral administration of terazosin. *J Pharmacol Exp Ther* **282**:891–898.
- Witte DG, Brune ME, Katwala SP, Milicic I, Stolarik D, Hui Y-H, Marsh KC, Kerwin JF Jr, Meyer MD, and Hancock AA (2002) Modeling of the relationships between pharmacokinetics and blockade of agonist-induced elevation of intraurethral pressure and mean arterial pressure in conscious dogs treated with terazosin, doxazosin, tamsulosin and fiduxosin. *J Pharmacol Exp Ther* **300**:XXX–XXX.
- Zhong H and Minneman KP (1999) α_1 -Adrenoceptor subtypes. *Eur J Pharmacol* **375**:261–276.

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