

# Preclinical Pharmacology of Fiduxosin, a Novel $\alpha_1$ -Adrenoceptor Antagonist with Uroselective Properties

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## ABSTRACT

Benign prostatic hyperplasia (BPH), common in aging males, is often treated with  $\alpha_1$ -adrenoceptor antagonists. To minimize hypotensive and other side effects, compounds with selective antagonist activity at  $\alpha_{1A}$ - and  $\alpha_{1D}$ - (compared with  $\alpha_{1B}$ -) adrenoceptors were evaluated that would block lower urinary tract  $\alpha_1$ -adrenoceptors in preference to cardiovascular  $\alpha_{1B}$ -adrenoceptors. Fiduxosin (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; ABT-980) was tested in radioligand binding assays, isolated tissue bioassays, intraurethral pressure (IUP) tests in isoflurane-anesthetized dogs, and blood pressure analyses in spontaneously hypertensive rats (SHR). Fiduxosin had higher affinity for cloned human  $\alpha_{1A}$ - (0.16 nM) and  $\alpha_{1D}$ -adrenoceptors (0.92 nM) in radioligand binding studies compared with  $\alpha_{1B}$ -adrenoceptors (25 nM) or in isolated tissue bioassays [ $pA_2$

values of 8.5–9.6 for  $\alpha_{1A}$ -receptors in rat vas deferens or canine prostate strips, 8.9 at  $\alpha_{1D}$ -adrenoceptors (rat aorta), compared with 7.1 at  $\alpha_{1B}$ -adrenoceptors (rat spleen)]. Furthermore, the compound antagonized putative  $\alpha_{1L}$ -adrenoceptors in the rabbit urethra ( $pA_2$  value of 7.58). Fiduxosin blocked epinephrine-induced increases in canine IUP (pseudo- $pA_2$  value of 8.12), eliciting only transient decreases in mean arterial blood pressure (MAP) in SHR. The area under the curve ( $AUC_{0\rightarrow 60}$  min) for the hypotensive response was dose related with a log index value for fiduxosin of 5.23, indicating a selectivity of 770-fold comparing IUP to MAP effects. Preferential antagonism of  $\alpha_{1A}$ - and  $\alpha_{1D}$ - versus  $\alpha_{1B}$ -adrenoceptors in vitro, blockade of putative  $\alpha_{1L}$ -sites in vitro, and selective effects on lower urinary tract function versus blood pressure in vivo by fiduxosin suggest the potential utility of this compound for the treatment of BPH.

Benign prostatic hyperplasia (BPH), a change in the size, composition, and function of the prostate gland, leads to obstruction of the bladder and urethra in middle-aged and elderly males. The enlarged prostate is composed of glandu-

lar epithelium and a large stromal component containing mostly smooth muscle (Shapiro and Lepor, 1991). Although the term BPH might suggest that symptoms arise exclusively from increased organ size causing mechanical obstruction of urine flow, no correlation between prostate size and symptom severity has been shown (Shapiro and Lepor, 1995). Rather, an important “dynamic” component to BPH results from alterations in sympathetic control of prostatic smooth muscle tone, mediated primarily through  $\alpha_1$ -adrenoceptor mecha-

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**ABBREVIATIONS:** BPH, benign prostatic hyperplasia; REC 15/2739, (N-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-3-methyl-4-oxo-2-phenyl-4H-1-benzopyran-8-carboxamide); Ro-70-0004, 3-(3-[4-(fluoro-2-(2,2,2-trifluoroethoxy)-phenyl)-piperazin-1-yl]-propyl)-5-methyl-1H-pyrimidine-2,4-dione mono hydrochloride monohydrate; 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); DMSO, dimethyl sulfoxide; PE, phenylephrine; IUP, intraurethral pressure; EPI, epinephrine; SHR, spontaneously hypertensive rats; MAP, mean arterial blood pressure; AUC, area under the curve;  $pED_{50}$ , negative logarithm of the molar dose of compound required to elicit a reduction in blood pressure for 60 min to a point midway between hypertensive and normotensive; ANOVA, analysis of variance;  $K_i$ , inhibition constant as a measure of drug affinity for a receptor, equivalent to the concentration of compound required to occupy 50% of receptors;  $pK_B$ , negative logarithm of the dissociation constant;  $pA_2$ , negative logarithm of the concentration of compound required to elicit a 2-fold shift of an agonist concentration-response curve in isolated tissues; 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); CL, confidence limit; B8805-033, [(±)-1,3,5-trimethyl-6-[[3-[4-((2,3dihydro-2-hydroxymethyl)-1,4-benzodioxin-5-yl)-1-piperazinyl]pro-pyl]amino]-2,4(1H,3H)-pyrimidin-one]; WB-4101, [2-(2,5-dimethoxyphenoxyethyl)-aminomethyl-1,4 benzodioxane]; BMY-7378, (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-1-7,9-dione); RWJ-38063, [N-(2-{4-[2-(methylethoxy)phenyl]-piperazinyl}ethyl)-2-(2-oxopiperadiny)acetamide]; RWJ-69736, [N-(3-{4-[2-(methylethoxy)phenyl]piperazinyl}propyl)-2-(2-oxopiperadiny)acetamide].

nisms, more than the “static” component related to the volume of glandular tissue. Over the past decade,  $\alpha_1$ -adrenoceptor antagonists have transformed BPH therapy from surgical to pharmacological intervention (Altwein, 1995), resulting in reduced adverse events (Barry and Roehrborn, 1997). However, several approved medications (e.g., terazosin and doxazosin) were originally identified as antihypertensive agents (for review, see Hancock, 1996), which may explain cardiovascular side effects associated with these compounds.

Since the discovery of subtypes of  $\alpha_1$ -adrenoceptors<sup>1</sup> and of the enrichment of the  $\alpha_{1A}$ -subtype in the human prostate gland (Price et al., 1993), investigators have pursued  $\alpha_{1A}$ -subtype-selective (Hancock, 1996) and, by extrapolation, “prostate-selective” antagonists. These compounds were designed to ameliorate BPH symptoms with fewer adverse effects (e.g., decreased blood pressure or postural hypotension and syncope) observed with nonselective  $\alpha_1$ -blockers. Quinazoline-type  $\alpha_1$ -antagonists (e.g., terazosin and doxazosin) with potent hypotensive and other cardiovascular effects have in some instances been shown to have slightly greater affinity for  $\alpha_{1B}$ - compared with  $\alpha_{1A}$ -adrenoceptors in functional and radioligand binding studies (Hancock, 1996), although this is not universally observed. Moreover, mice deficient in the  $\alpha_{1B}$ -adrenoceptor show diminished blood pressure responses to phenylephrine injection compared with homozygous controls (Cavalli et al., 1997). These observations suggest that  $\alpha_{1B}$ -adrenoceptors are more important for blood pressure regulation, and that compounds having reduced activity at  $\alpha_{1B}$ -sites compared with other  $\alpha_1$ -adrenoceptors would be expected to cause fewer cardiovascular side effects than classical  $\alpha_1$ -antagonists (Take et al., 1998), supporting the concept that an  $\alpha_{1A}$ -selective compound would be useful in BPH (Hancock et al., 1998a). Tamsulosin causes fewer hypotensive side effects in clinical practice (de Mey, 1998) and in animal studies (Hancock et al., 1998a,b), despite only moderate differences ( $\leq 20$  fold) in affinity at  $\alpha_{1A}$ - compared with either  $\alpha_{1B}$ - or  $\alpha_{1D}$ -adrenoceptors (Hancock, 1996). However, several highly selective  $\alpha_{1A}$ -antagonists intended to be uroselective, including REC 15/2739 (Leonardi et al., 1997) and Ro-70-0004 (Williams et al., 1999), failed to improve both voiding and irritative symptoms in the clinic, such that the hypothesis of  $\alpha_{1A}$ -selectivity correlating to uroselectivity remains unproven. Recent observations suggest that blockade of  $\alpha_{1A}$ -adrenoceptors may promote relief of voiding symptoms but not the irritative and filling symptoms in BPH (Michel et al., 2000). In contrast,  $\alpha_{1D}$ -adrenoceptors may have a key role for irritative and filling symptoms consistent with detrusor instability (Broten et al., 1998; Michel et al., 2000; Schwinn and Michelotti, 2000), a frequent and major component of BPH symptomatology (Rosier et al., 1995). In a rat model of bladder obstruction, reversal of the ratio of detrusor  $\alpha_{1A}$ - to  $\alpha_{1D}$ -adrenoceptors (73:25) was seen after 6 weeks of urethral obstruction (22:75) (Hampel et al., 2000). Spinal or supraspinal  $\alpha_{1D}$ -adrenoceptors (Smith et al., 1999; Michel et al., 2000) may also be important to control bladder function. Thus, a selective  $\alpha_{1A}$ -/ $\alpha_{1D}$ -antagonist, relative to  $\alpha_{1B}$ -receptors, may have the potential to treat both voiding

and filling symptoms of BPH without the hemodynamic liabilities of currently used agents.

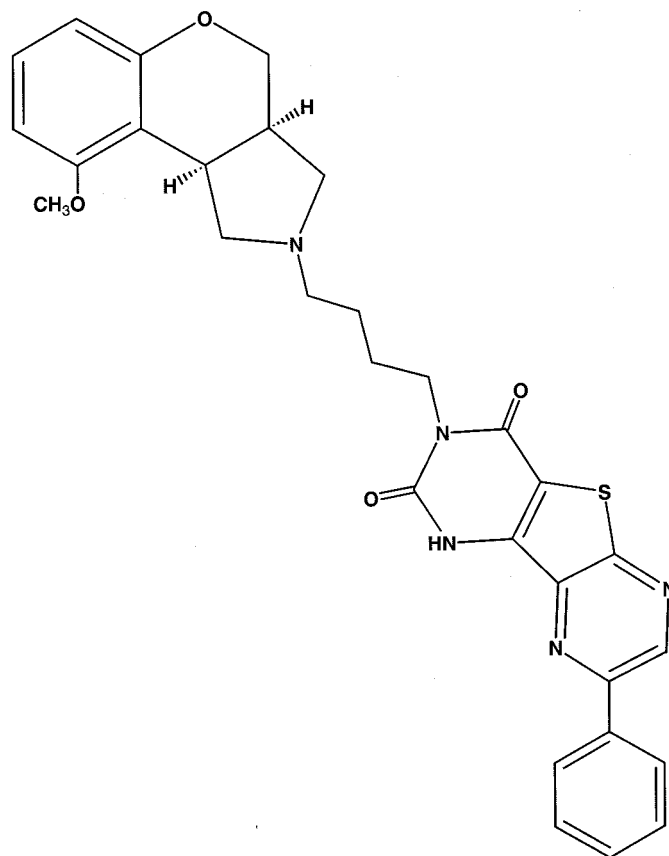
A confounding issue arises from studies of  $\alpha_{1L}$ -adrenoceptors, which demonstrate low affinity for antagonists such as prazosin in some studies (Ford et al., 1993; Muramatsu et al., 1994; Leonardi et al., 1997; Testa et al., 1997). Because signal transduction (Chang et al., 1998) and radioligand binding (Ford et al., 1997; Chang et al., 1998; Mason et al., 1998) assays show low affinity of some compounds at  $\alpha_{1A}$ -adrenoceptors, the  $\alpha_{1L}$ -site may represent an altered affinity state of the  $\alpha_{1A}$ -subtype (Ford et al., 1997) or an artifact (Narayan and Tewari, 1998).

In this article, the initial in vitro and in vivo pharmacology of fiduxosin (ABT-980; Fig. 1), a novel  $\alpha_1$ -antagonist with preferential affinity for those sites that may be important for BPH pharmacotherapy, namely,  $\alpha_{1A}$ -,  $\alpha_{1D}$ -, and putative  $\alpha_{1L}$ -adrenoceptors, with low potency at  $\alpha_{1B}$ -adrenoceptors, is described. The goal of these studies was to determine whether this compound might represent a “uroselective” antagonist.

## Experimental Procedures

**Radioligand Binding Assays.** Radioligand binding assays were performed as described (Hancock et al., 1998b), by using recombinant human  $\alpha_1$ -adrenoceptors expressed in mouse fibroblast cells (LTK<sup>-</sup>). Membranes were prepared from confluent cells of stable single cell clones as previously described (Hancock et al., 1998b).

Radioligand binding was determined in tubes containing 0.05 ml



**Fig. 1.** Chemical structure of fiduxosin. Fiduxosin (ABT-980) is defined chemically as (3-[4-((3a*R*,9b*R*)-*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]thi-eno [3,2-*d*]pyrimidine-2,4(1*H*,3*H*)-dione). The hydrochloride salt was used for experimental testing.

<sup>1</sup> In this article, nomenclature used to differentiate among the subtypes of  $\alpha_1$ -adrenoceptors uses uppercase subscripted letters to describe tissue-sourced receptors and lowercase subscripted letters to define cloned receptors (Bylund et al., 1994).

of water (total binding); 10  $\mu$ M final concentration of phentolamine (nonspecific binding) or compound of interest; 0.45 ml [ $^3$ H]prazosin, approximately 200 pM; and 0.5 ml of receptor preparation (generally 0.83-mg wet weight or approximately 0.1 mg of protein/assay tube) in 50 mM Tris-HCl, pH 7.4; and samples were incubated 60 min at 25°C. Under these conditions, less than 10% of added radioligand was bound to the receptors. Fiduxosin was dissolved in 10% DMSO and all serial dilutions were performed manually because of the tendency of this compound to adhere to automated pipetting devices. Assays were terminated by filtration under vacuum through Whatman GF/B filters and data analyzed as previously described (Hancock et al., 1998b).

#### Isolated Tissue Bioassays for Functional Activity in Vitro.

Rat vas deferens, spleen, or aorta or canine prostate glands (male beagles aged >3 years) were studied as previously described (Hancock et al., 1998b). For isolated rabbit urethra, female New Zealand White rabbits (1.75–3.5 kg) were sacrificed by means of a 0.5-ml/kg i.p. injection of pentobarbital solution (Somlethal; J. A. Webster Inc., Sterling, MA). The urethra was removed with the urinary bladder and immediately placed into Krebs-Henseleit buffer of the following composition: 119 mM NaCl, 4.7 mM KCl, 1.2 mM  $\text{KH}_2\text{PO}_4$ , 2.5 mM  $\text{CaCl}_2$ , 0.01 mM  $\text{K}_2\text{EDTA}$ , 20 mM  $\text{NaHCO}_3$ , 1.5 mM  $\text{Mg}_2\text{SO}_4$ , 11 mM dextrose, and 0.004 mM propranolol. The urethra was separated from the bladder, cut into four tissue rings approximately 3 to 4 mm in width, and subsequently fixed between a stationary glass rod and a force-displacement transducer as previously described for other isolated tissues (Hancock et al., 1998b) at a basal preload of 1.0 g. After equilibration with intermittent rinsing for 45 to 60 min, tissues were primed with 80 mM KCl, rinsed to basal tension and stimulated with 10  $\mu$ M phenylephrine (PE). After 60 min equilibration, a control (PE) cumulative concentration response was determined for each tissue. After a 75-min washout, agonist concentration-response curves were generated in the presence and absence of test compounds and the data analyzed as described previously (Hancock et al., 1998b). Fiduxosin was dissolved in 100% DMSO with subsequent dilution in DMSO. Because test drugs were diluted 1000-fold in the organ bath, DMSO had no effect on tissue responses. For studies with fiduxosin, tissues were rinsed after the initial agonist concentration-response curve, and fiduxosin was added to the tissue bath and allowed to equilibrate for 4 h. After each hour, fresh buffer was applied and fiduxosin replaced in the tissue bath.

**Measurement of IUP in Anesthetized Dogs.** Experimental procedures described below were reviewed and approved by the Institutional Animal Care and Use Committee of Abbott Laboratories. Intraurethral pressure (IUP) responses to i.v. epinephrine (EPI) were recorded by inserting a balloon catheter into the prostatic urethra through the penis of isoflurane-anesthetized dogs as previously described (Hancock et al., 1998a). Briefly, a lubricated 7F Swan-Ganz balloon catheter was inserted into the urethral orifice and advanced until the balloon tip was placed within the bladder. The balloon was inflated with 1 ml of room air and the catheter slowly withdrawn just past the first resistance felt at the bladder neck, placing the balloon within the prostatic urethra. The balloon port of the catheter was connected to an Abbott Transpac pressure transducer (42556-01; Abbott Laboratories, North Chicago, IL) interfaced to a data acquisition system for measurement of IUP. EPI and test compounds were administered through a cannula in the cephalic vein. In male dogs greater than 2 years of age, EPI causes robust, dose-dependent increases in IUP between 10 and 50 mm Hg for doses of 10 to 100 nmol/kg i.v., respectively (Hancock et al., 1998a).

**Blood Pressure Measurements in Conscious SHR.** MAP (mm Hg) was measured in rats as previously described (Hancock et al., 1998b). In brief, rats (15–20 weeks in age) were anesthetized with methoxyflurane (Abbott Laboratories) while the left femoral artery and vein were catheterized using polyethylene 50 tubing for measurement of MAP and compound administration, respectively. The catheters were filled with heparinized 0.9% saline (10 U/ml), passed

subcutaneously to a point behind the neck, exteriorized, and the arterial catheter connected to a Gould Statham P23Dd pressure transducer interfaced to a Grass polygraph. MAP was determined on-line by using a BUXCO cardiovascular analyzer (BUXCO Electronics, Sharon, CT). After 2 to 3 h of recovery from surgery and a 30-min predose control period, each rat was given one dose of a test antagonist i.v. and MAP was monitored for an additional 2.5 h. The percentage of change from an average predose control value was calculated for each time point and the area under the hypotensive response curve from 0 to 60 min postdosing ( $T_{60}$  AUC) was determined using a trapezoidal rule integration of that data set. Periodically rats were exposed to 90° head-up tilt either before or after compound administration to determine the potential for interference with reflex control of blood pressure during postural events (Hancock et al., 1998b).

**Data Analysis and Statistical Procedures.** One-way analysis of variance (ANOVA; Snedecor and Cochran, 1967) of individual  $K_i$  values was used to compare compound potencies in receptor binding assays by using RS/1 (BBN Software Products, Cambridge, MA) with statistical significance indicated by a  $P$  value < 0.05. For isolated tissue bioassays, individual  $pK_B$  values of each compound were determined and compared across tissue types by using ANOVA procedures in RS/1. For anesthetized dog experiments, the effects of antagonists on EPI-induced responses were determined as shifts in the agonist dose-response curves and data were analyzed according to previously described methods (Hancock et al., 1998a). The standard error and S.E.M. of the  $pA_2$  values were determined using methods previously described (Hancock et al., 1998b).

To quantify the magnitude and duration of hypotensive responses in SHR, the area under the curve between 0 and 60 min for the hypotensive response of each dose of antagonist was determined. By analyzing AUC values for each animal at each dose of compound using ALLFIT (Hancock et al., 1998b), an estimate of the dose of compound required to reduce MAP of SHR to the midpoint toward normotensive levels was obtained. The negative logarithms of these doses were compared on a molar basis ( $pED_{50}$  values) to determine the relative potencies of antagonists as antihypertensive agents by using either  $F$  tests in ALLFIT, or by using ANOVA after RS/1 procedures with statistical significance indicated by a  $P$  value < 0.05. For comparison of orthostatic hypotensive responses in SHR, paired  $t$  tests were used to determine significant responses to tilt at each time point, comparing the blood pressure effect of the compound alone with any additive effect of tilt.

**Materials.** Fiduxosin (ABT-980), A-131701, prazosin, terazosin, doxazosin, alfuzosin, tamsulosin, and REC 15/2739 were synthesized at Abbott Laboratories. [ $^3$ H]Prazosin (75–80 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). L-Epinephrine and L-PE were purchased from Sigma Chemical (St. Louis, MO). Phentolamine was obtained from Novartis Pharmaceuticals (Summit, NJ).

## Results

**Radioligand Binding Assays.** The potency of fiduxosin in radioligand binding assays is compared with the predominant clinically used  $\alpha_1$ -antagonists terazosin and tamsulosin at cloned human  $\alpha_1$ -adrenoceptors (Table 1). The affinity of fiduxosin for  $\alpha_{1a}$ -,  $\alpha_{1b}$ -, and  $\alpha_{1d}$ -adrenoceptors was 0.160 nM (0.096–0.267, 95% CL), 24.9 nM (1.92–32.3), and 0.920 nM (0.659–1.28), respectively ( $pK_i$  values of 9.80, 7.60, and 9.04, respectively; Table 1). Fiduxosin was approximately 155-fold more potent at  $\alpha_{1a}$ -adrenoceptors than at  $\alpha_{1b}$ -adrenoceptors, but was only 6-fold more potent at  $\alpha_{1a}$ - than at  $\alpha_{1d}$ -adrenoceptors. In contrast, terazosin displayed minor potency differences at the three receptors [ $K_i$  = 1.81 nM (1.45–2.26, 95% CL), 1.16 nM (0.79–1.70), and 0.667 nM (0.549–0.810) for

TABLE 1

Comparative radioligand binding potencies of fiduxosin and standard  $\alpha_1$ -adrenoceptor antagonists at subtypes of  $\alpha_1$ -receptors

Membranes containing  $\alpha_1$ -adrenoceptors were incubated with various concentrations of test agents in the presence of [ $^3$ H]prazosin as described under *Experimental Procedures*. Values are the negative log of geometric means of nanomolar affinity ( $pK_i$ ) and the S.E.M. of  $n$  separate determinations.

Compound	Receptor		
	Human $\alpha_{1a}$	Human $\alpha_{1b}$	Human $\alpha_{1d}$
Fiduxosin <sup>a</sup>	9.80 ( $\pm 0.096$ ) [9] <sup>b</sup>	7.60 ( $\pm 0.049$ ) [9]	9.04 ( $\pm 0.063$ ) [9]
Terazosin <sup>c</sup>	8.74 ( $\pm 0.046$ ) [19]	8.94 ( $\pm 0.076$ ) [12]	9.18 ( $\pm 0.038$ ) [12]
Tamsulosin <sup>d</sup>	10.54 ( $\pm 0.041$ ) [5]	9.22 ( $\pm 0.111$ ) [7]	10.24 ( $\pm 0.039$ ) [4]

<sup>a</sup> Fiduxosin potency order was  $\alpha_{1a} > \alpha_{1d} > \alpha_{1b}$ , with each difference of statistical significance (ANOVA,  $P < 0.05$ ).

<sup>b</sup> Values shown:  $pK_i$  ( $\pm$ S.E.M.) [ $n$ ].

<sup>c</sup> Terazosin potency order was  $\alpha_{1d} > \alpha_{1b} > \alpha_{1a}$ , with only the potency difference between  $\alpha_{1d}$  and  $\alpha_{1a}$  of statistical significance (ANOVA,  $P < 0.05$ ).

<sup>d</sup> Tamsulosin potency order was  $\alpha_{1a} \approx \alpha_{1d} > \alpha_{1b}$ , with only the lower potency at  $\alpha_{1b}$ -sites of statistical significance (ANOVA,  $P < 0.05$ ).

$\alpha_{1a}$ -,  $\alpha_{1b}$ -, and  $\alpha_{1d}$ -adrenoceptors, respectively ( $pK_i$  values of 8.74, 8.94, and 9.18; Table 1), with statistical significance achieved only in comparing the  $\alpha_{1a}$ - and  $\alpha_{1d}$ -potency values. Tamsulosin was ~20-fold  $\alpha_{1a}$ -selective compared with  $\alpha_{1b}$ -adrenoceptors [ $K_i = 0.029$  nM (0.022–0.038 95% CL) and 0.602 nM (0.328–1.1) for  $\alpha_{1a}$ - and  $\alpha_{1b}$ -adrenoceptors, respectively], but nonselective compared with  $\alpha_{1d}$ -receptors [ $K_i = 0.058$  nM (0.044–0.077) for  $\alpha_{1d}$ -adrenoceptors] as previously reported (Kenny et al., 1994; Hancock, 1996). (Corresponding  $pK_i$  values for tamsulosin were 10.54, 9.22, and 10.24, respectively; Table 1.) Fiduxosin displayed low affinity for other adrenoceptors, including cloned human  $\alpha_{2a}$ - [92 nM (52–160, 95% CL)] and  $\alpha_{2c}$ -adrenoceptors [22 nM (12–42, 95% CL)] and rat neonatal lung  $\alpha_{2b}$ -adrenoceptors [21 nM (12–38, 95% CL)], as well as  $\beta$ -adrenoceptors (2–5  $\mu$ M; data not shown). Fiduxosin also had low affinity for 5HT1A receptors in rat cortex [29 nM (18–47, 95% CL)] compared with its affinity at  $\alpha_{1a}$ -adrenoceptors (0.160 nM). Thus, fiduxosin is approximately 180-fold selective for  $\alpha_{1a}$ - compared with 5HT1A receptors, unlike a number of compounds from the orthomethoxy piperazine class of compounds (e.g., BMY 7378, 5-methyl-urapidil), which have higher potency for 5HT1A compared with  $\alpha_{1a}$ -adrenoceptors (Hancock, 1996). Similarly,

TABLE 2

Comparative functional antagonistic potencies of fiduxosin and standard  $\alpha_1$ -adrenoceptor antagonists at  $\alpha_1$ -adrenoceptors in isolated smooth muscle in vitro

Isolated smooth muscle strips or rings were incubated in tissue baths in the presence or absence of various concentrations of fiduxosin and standard  $\alpha_1$ -adrenoceptor antagonists, as described under *Experimental Procedures*.

Compound	Tissue and Receptor Subtype				
	Rat Vas Deferens $\alpha_{1A}$	Canine Prostate $\alpha_{1A}$	Rabbit Urethra $\alpha_{1L}$	Rat Spleen $\alpha_{1B}$	Rat Aorta $\alpha_{1D}$
Fiduxosin <sup>a</sup>					
$pA_2$ ( $\pm$ S.E.M.)	9.62 ( $\pm 0.44$ )	8.51 ( $\pm 0.23$ )	7.58 ( $\pm 0.19$ )	7.08 ( $\pm 0.07$ )	8.92 ( $\pm 0.86$ )
Slope ( $\pm$ S.E.M.)	0.83 ( $\pm 0.07$ )	0.80 ( $\pm 0.05$ )	0.92 ( $\pm 0.13$ )	0.90 ( $\pm 0.05$ )	0.78 ( $\pm 0.08$ )
[ $n$ ]	[18]	[20]	[15]	[14]	[20]
Terazosin <sup>b</sup>					
$pA_2$ ( $\pm$ S.E.M.)	8.04 ( $\pm 0.45$ )	7.44 ( $\pm 0.24$ )	6.77 ( $\pm 0.30$ )	8.60 ( $\pm 0.46$ )	8.65 ( $\pm 0.29$ )
Slope ( $\pm$ S.E.M.)	0.83 ( $\pm 0.17$ )	0.79 ( $\pm 0.09$ )	0.99 ( $\pm 0.07$ )	0.94 ( $\pm 0.14$ )	0.99 ( $\pm 0.13$ )
[ $n$ ]	[12]	[35]	[13]	[12]	[9]
Tamsulosin <sup>c</sup>					
$pA_2$ ( $\pm$ S.E.M.)	9.47 ( $\pm 0.21$ )	9.54 ( $\pm 0.17$ )	8.86 ( $\pm 0.22$ )	9.69 ( $\pm 0.44$ )	10.6 ( $\pm 0.43$ )
Slope ( $\pm$ S.E.M.)	1.06 ( $\pm 0.14$ )	1.12 ( $\pm 0.08$ )	1.41 ( $\pm 0.26$ )	0.84 ( $\pm 0.15$ )	0.94 ( $\pm 0.11$ )
[ $n$ ]	[22]	[20]	[8]	[16]	[10]

<sup>a</sup> One-way analysis of variance indicated the following potency order of statistically significant differences for fiduxosin: rat vas deferens  $>$  rat spleen  $\approx$  canine prostate  $>$  rabbit urethra  $>$  rat spleen  $\alpha_1$ -adrenoceptor antagonism.

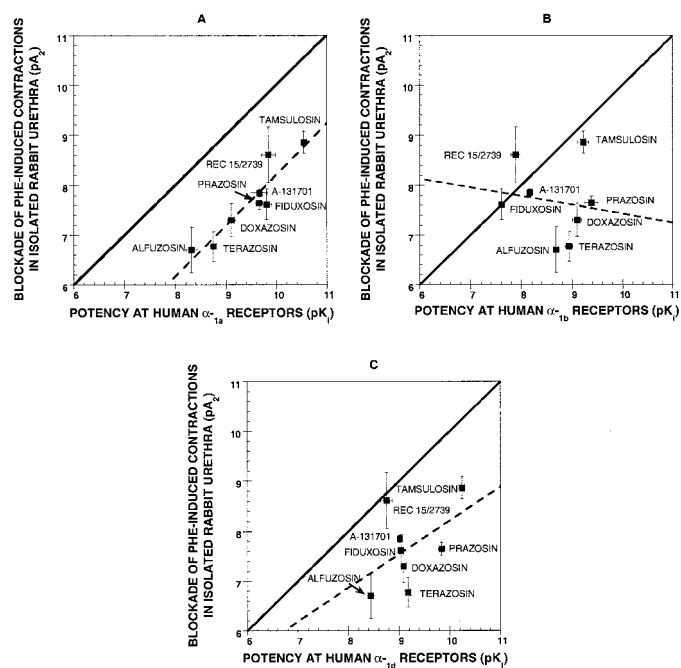
<sup>b</sup> One-way analysis of variance indicated the following potency order of statistically significant differences for terazosin: rat aorta  $\approx$  rat spleen  $>$  rat vas deferens  $>$  canine prostate  $>$  rabbit urethra  $\alpha_1$ -adrenoceptor blockade.

<sup>c</sup> One-way analysis of variance indicated the following potency order of statistically significant differences for tamsulosin: rat aorta  $>$  canine prostate  $>$  rabbit urethra, but canine prostate  $\approx$  rat vas deferens  $\approx$  rat spleen and rat vas deferens  $\approx$  rat spleen  $\approx$  rabbit urethra  $\alpha_1$ -adrenoceptor antagonism.

the 5-methyl-urapidil analog B8805-033 (Eltze et al., 2001) is at least 10-fold more potent at 5HT1A compared with  $\alpha_{1a}$ -adrenoceptors. Likewise, the classical  $\alpha_{1a}$ -selective compound WB-4101 (only 3-fold  $\alpha_{1a}$ -selective) has high affinity for 5HT1A receptors (Hancock, 1996). These  $\alpha_1$ -antagonists with high 5HT1A affinity represent a challenge in in vivo experiments, because of the potential cardiovascular effects of 5HT1A stimulation on central sympatho-inhibitory pathways (Gillis et al., 1989). Because of the lack of high affinity for 5HT1A sites by fiduxosin, no functional analysis of agonist or antagonistic activity was attempted for this compound.

**Isolated Tissue Bioassays for Functional Activity in Vitro.** In agreement with radioligand binding data, fiduxosin was between 27- and 350-fold more potent as an antagonist of  $\alpha_{1A}$ -adrenoceptors in rat vas deferens or canine prostate compared with  $\alpha_{1B}$ -adrenoceptors in rat spleen (Table 2). However, fiduxosin was intermediate in potency at  $\alpha_{1D}$ -adrenoceptors in the rat aorta compared with  $\alpha_{1A}$ -adrenoceptors in rat vas deferens and canine prostate (Table 2). Fiduxosin was a competitive antagonist at each receptor, as determined on the basis of parallel shifts to concentration-response curves, with slopes of the Schild plots not significantly different from unity and no inhibition of maximal agonist-induced contractile responses with increasing concentrations of compound (data not shown). For unknown reasons, fiduxosin demonstrates weaker antagonism of canine prostatic  $\alpha_{1A}$ - compared with  $\alpha_{1A}$ -adrenoceptors in rat vas deferens. This is a phenomenon observed with some, but not all, compounds and may simply be a reflection of the heterogeneity of the tissues, differences in protein binding or distribution among compounds, and inherent variability of the bioassay, or related to the  $\alpha_{1L}$ -phenotype observed with some compounds in assays of prostatic function. For example, like fiduxosin, WB-4101, doxazosin, and L-terazosin are approximately 10-fold less potent at canine prostatic compared with rat vas deferens  $\alpha_{1A}$ -adrenoceptors (data not shown). In contrast, prazosin, R-terazosin, and (-)-WB-4101 are less potent at the canine receptors by only 2- to 4-fold (data not shown), similar to tamsulosin and terazosin (Table 1).

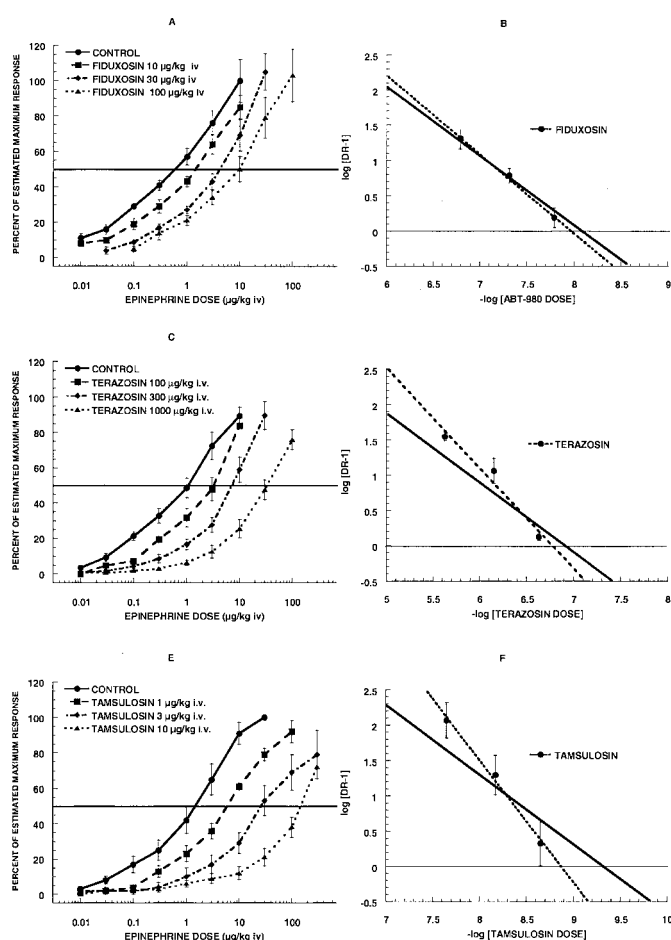
In the rabbit urethra, fiduxosin antagonized competitively



**Fig. 2.** Correlation analysis of antagonism of rabbit urethral  $\alpha_1$ -adrenoceptors to compound affinities at cloned human  $\alpha_1$ -adrenoceptor subtypes. Blockade of PE-induced contractions of rabbit urethra was quantified as  $pA_2$  values (Table 2) and compared with radioligand binding affinities (Table 1; Hancock et al., 1998b) for cloned human  $\alpha_{1A}$ - (A),  $\alpha_{1B}$ - (B), and  $\alpha_{1D}$ -adrenoceptors (C), respectively. The correlation coefficients were 0.93 for  $\alpha_{1A}$ -adrenoceptors, with a slope of 1.02. For  $\alpha_{1B}$ -adrenoceptors the correlation coefficient was 0.15, with a slope of  $-0.17$ , whereas for  $\alpha_{1D}$ -adrenoceptors the correlation coefficient was 0.50, with a slope of 0.67.

PE-induced responses with a  $pA_2$  value of 7.58, intermediate between the high potency of tamsulosin and the weaker affinity of terazosin (Table 2) and several other  $\alpha_1$ -antagonists (Fig. 2). The low potency of prazosin and the other quinazolines to block PE-induced contractions in the urethra model is consistent with these receptors being of the  $\alpha_{1L}$ -type (Leonardi et al., 1997; Testa et al., 1997; Van der Graaf et al., 1997). However, the potency order of structurally diverse antagonists in this model was highly correlated to the potency of these compounds at  $\alpha_{1A}$ -adrenoceptors in radioligand binding studies, but not with either the  $\alpha_{1B}$ - or  $\alpha_{1D}$ -receptor (Fig. 2). As predicted from radioligand binding data, terazosin was essentially equipotent as an antagonist of  $\alpha_{1B}$ - and  $\alpha_{1A}$ -adrenoceptors (Table 2) in isolated tissues, although it was less potent in rabbit urethra. Tamsulosin, as previously reported (Hancock, 1996), inhibited maximal responses in the rat vas deferens consistent with a noncompetitive blockade of some  $\alpha_{1A}$ -adrenoceptors, and was not selective for  $\alpha_{1A}$ - compared with  $\alpha_{1B}$ -adrenoceptors in functional tests in vitro (Hancock et al., 1998b).

**Measurement of IUP in Dogs.** Fiduxosin, administered at doses of 30, 100, and 300  $\mu\text{g}/\text{kg}$  i.v. (0.051, 0.168, and 0.507  $\mu\text{mol}/\text{kg}$  i.v.) antagonized IUP responses to i.v. EPI in anesthetized dogs (Fig. 3A), presumably via blockade of  $\alpha_1$ -adrenoceptor-mediated smooth muscle contraction within the prostatic stroma (Brune et al., 1995). Fiduxosin caused rightward shifts to the EPI dose-response curve, yielding an in vivo pseudo- $pA_2$  value of  $8.12 \pm 0.17$  (Fig. 3B; Table 3). Comparable data for terazosin and tamsulosin are illustrated in Fig. 3, C to F, and summarized in Table 3. The rank order



**Fig. 3.** Effects of  $\alpha_1$ -adrenoceptor antagonists on IUP in anesthetized dogs. Fiduxosin (A and B), terazosin (C and D), and tamsulosin (E and F) were administered as described under *Experimental Procedures* to anesthetized dogs in various doses, followed by intravenous administration of epinephrine over a range of doses. Intraurethral pressure was measured as described under *Experimental Procedures*. A, C, and E, shifts of the epinephrine dose-response curve caused by the different doses of the  $\alpha_1$ -antagonists. Solid lines indicate control epinephrine dose responses in the absence of antagonist. Increasing doses of fiduxosin of 10, 30, and 100  $\mu\text{g}/\text{kg}$  i.v.; terazosin of 100, 300, or 1000  $\mu\text{g}/\text{kg}$  i.v.; or tamsulosin of 1, 3, and 10  $\mu\text{g}/\text{kg}$  i.v. elicited dose-dependent rightward shifts of the epinephrine dose-response relationship. B, D, and F, Schild plots of the shifts of the dose-response curves to generate the pseudo- $pA_2$  values listed in Table 3. Dashed lines are the regression lines of the data sets, whereas the solid lines indicate the slope of the Schild line constrained to equal 1. For fiduxosin, the estimated  $pA_2$  value was 8.12, but 8.16 when the slope was constrained to 1. Values for terazosin (6.67 and 6.90) and tamsulosin (8.87 and 9.36) were also obtained in the absence and presence, respectively, of the constraint. The difference was greatest for tamsulosin because the slope of the Schild plot was 1.76 (Table 3).

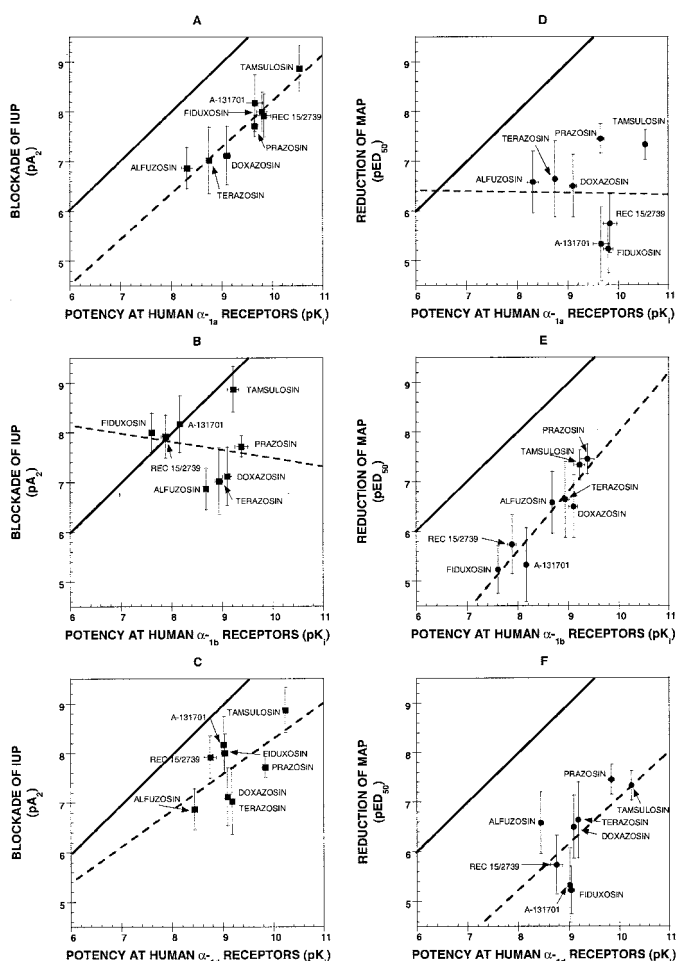
of potencies for blocking EPI-induced pressor responses in the dog for fiduxosin and several other  $\alpha_1$ -antagonists was similar to the potency order of these compounds at isolated canine prostatic strips, rabbit urethral smooth muscle, or rat vas deferens (Table 2), less similar at  $\alpha_{1D}$ -adrenoceptors in rat aorta, and distinct from the potency order at rat spleen  $\alpha_{1B}$ -adrenoceptors (Table 2). The potency order for blockade of IUP responses was also highly correlated to compound affinities in receptor binding assays of cloned human  $\alpha_{1A}$ -adrenoceptors for fiduxosin and several chemical classes of  $\alpha_1$ -antagonists (Fig. 4A), but lesser correlations were observed for either  $\alpha_{1B}$ - or  $\alpha_{1D}$ -receptors (Fig. 4, B and C).

TABLE 3

Comparative antagonistic potencies of fiduxosin and standard  $\alpha_1$ -adrenoceptor antagonists at  $\alpha_1$ -adrenoceptors in canine prostatic urethra and SHR vascular smooth muscle in vivo

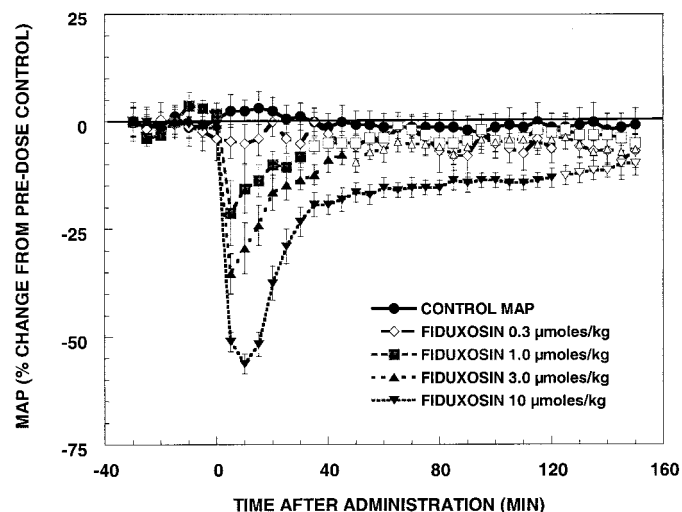
Dogs ( $n \geq 4$  per compound) were anesthetized with methoxyflurane, and prostatic urethral responses to EPI were determined as described under *Experimental Procedures*. Potencies of  $\alpha_1$ -antagonists were determined from Schild analysis and expressed as  $pA_2$  values. SHR ( $n \geq 3$  per compound) were administered test agents i.v., and potencies ( $pED_{50}$  doses) to reduce blood pressure were determined as described under *Experimental Procedures*. The selectivity ratio was calculated as the antilog of the difference between the  $pA_2$  value versus EPI in the isoflurane-anesthetized canine and the  $pED_{50}$  in the SHR.

Compound	Isoflurane-Anesthetized Dog, IUP vs. i.v. EPI $pA_2 \pm$ S.E.M. (Slope $\pm$ S.E.M.)	SHR, MAP $pED_{50} \pm$ S.E.M. (Slope $\pm$ S.E.M.)	Selectivity Ratio, Antilog of ( $pA_2 - pED_{50}$ ) (IUP-MAP)
Fiduxosin	$8.12 \pm 0.17$ ( $1.1 \pm 0.19$ )	$5.23 \pm 0.48$ ( $0.83 \pm 0.14$ )	770
Terazosin	$6.67 \pm 0.18$ ( $1.44 \pm 0.42$ )	$6.64 \pm 0.76$ ( $0.85 \pm 0.17$ )	1.1
Tamsulosin	$8.87 \pm 0.19$ ( $1.76 \pm 0.45$ )	$7.33 \pm 0.30$ ( $0.37 \pm 0.15$ )	35



**Fig. 4.** Correlation analysis of antagonism of canine IUP and SHR MAP responses to compound affinities at cloned human  $\alpha_1$ -adrenoceptor subtypes. Blockade of EPI-induced elevations of IUP was quantified as pseudo- $pA_2$  values (Table 2) and compared with radioligand binding affinities (Table 1; Hancock et al., 1998b) for cloned human  $\alpha_{1a}$ - (A),  $\alpha_{1b}$ - (B), or  $\alpha_{1d}$ -adrenoceptors (C), respectively. The correlation coefficients were 0.96 for  $\alpha_{1a}$ -adrenoceptors, with a slope of 0.92. For  $\alpha_{1b}$ -adrenoceptors the correlation coefficient was 0.16, with a slope of  $-0.17$ , whereas for  $\alpha_{1d}$ -adrenoceptors the correlation coefficient was 0.62 with a slope of 0.72. Hypotensive responses in SHR were quantified as  $pED_{50}$  values (Table 2) and were compared with radioligand binding affinities (Table 1; Hancock et al., 1998b) for cloned human  $\alpha_{1a}$ - (D),  $\alpha_{1b}$ - (E), or  $\alpha_{1d}$ -adrenoceptors (F), respectively. The correlation coefficients were 0.016 for  $\alpha_{1a}$ -adrenoceptors, with a slope of  $-0.019$ . For  $\alpha_{1b}$ -adrenoceptors the correlation coefficient was 0.94, with a slope of 1.2, whereas for  $\alpha_{1d}$ -adrenoceptors the correlation coefficient was 0.63 with a slope of 0.92.

**Blood Pressure Measurements in Conscious Spontaneously Hypertensive Rats.** Fiduxosin, administered to SHR at doses of 0.3, 1, 3, or 10  $\mu\text{mol/kg}$  i.v. (178, 592, 1780,



**Fig. 5.** Effects of fiduxosin on MAP in conscious SHR. Male SHR were briefly anesthetized for arterial and venous catheterization as described under *Experimental Procedures*. Hypotensive effects of fiduxosin [vehicle control, 0.3, 1, 3, or 10  $\mu\text{mol/kg}$  i.v. (0, 178, 592, 1780, and 5920  $\mu\text{g/kg}$  i.v.)] are shown, with mean initial MAP values of 155, 175, 160, 170, and 174 mm Hg, respectively, for groups of four to seven SHR. For rats treated with fiduxosin, filled symbols represent statistically significant hypotension, whereas open symbols do not differ from controls (filled circles).

and 5920  $\mu\text{g/kg}$  i.v.), elicited transient effects on blood pressure, particularly at doses of 0.3, 1, or 3  $\mu\text{mol/kg}$  i.v. (Fig. 5), with no effect of the lowest dose on MAP compared with vehicle and transient hypotension with doses between 1 and 3  $\mu\text{mol/kg}$  i.v. (Fig. 5). Only when the dose of fiduxosin was increased to 10  $\mu\text{mol/kg}$  i.v. was the hypotensive effect sustained (Fig. 5), although the decrease in MAP even at the highest dose was considerably less than the hypotension observed with nonselective  $\alpha_1$ -antagonists at equivalent or considerably lower doses (Hancock et al., 1998b). For example, prazosin, doxazosin, terazosin, and alfuzosin all decreased blood pressure by greater than 40% at doses of 1 to 3  $\mu\text{mol/kg}$  i.v., generally for the entire observation period of 150 min (Hancock et al., 1998b). Note that the lowest dose of fiduxosin tested in SHR (178  $\mu\text{g/kg}$  i.v.) exceeds the highest dose tested in the canine IUP model (100  $\mu\text{g/kg}$  i.v.), demonstrating that doses of fiduxosin that robustly block IUP responses in the dog elicit no significant hypotensive effect in SHR.

Using a hypotensive index described under *Experimental Procedures*, the potency of fiduxosin ( $\text{mol/kg}$  i.v.) was converted to a  $pED_{50}$  value of 5.23 (Table 3). We have previously determined (Hancock et al., 1998b) that if compounds are assessed using either a 30-min AUC or the peak hypotensive effect, the rank order of potencies is virtually identical, sug-

gesting that the 60-min AUC values provide a useful quantification of MAP effects. The pED<sub>50</sub> value observed for fiduxosin is considerably higher than that for typical  $\alpha_1$ -adrenoceptor antagonists (Hancock et al., 1998b). For example, the pED<sub>50</sub> values for prazosin (7.45), doxazosin (6.50), terazosin (6.64), alfuzosin (6.58), and tamsulosin (7.33) were 10- to 100-fold lower (Hancock et al., 1998b), consistent with the greater hypotensive effect of these  $\alpha_1$ -blockers. If the potency (pA<sub>2</sub>) of fiduxosin to block IUP effects in the canine is compared with its potency (pED<sub>50</sub>) to lower blood pressure, a relative index of selectivity of almost three orders of magnitude (770-fold) is obtained, making fiduxosin the most selective of the compounds for IUP compared with MAP effects (Hancock et al., 1998b). In addition, the relative hypotensive potency of fiduxosin and other  $\alpha_1$ -adrenoceptor antagonists was highly correlated to their affinity at  $\alpha_{1b}$ -adrenoceptors in radioligand binding studies (Fig. 4E), but less well correlated with the potency of these compounds at either  $\alpha_{1a}$ - or  $\alpha_{1d}$ -adrenoceptors (Fig. 4, D and F, respectively). It is noteworthy that fiduxosin is the least potent compound of those tested in measures of hypotensive efficacy (Fig. 4, D–F) and affinity for the  $\alpha_{1b}$ -adrenoceptor, consistent with the concept that hypotensive effects of  $\alpha_1$ -adrenoceptor antagonists primarily result from their antagonism of  $\alpha_{1b}$ -adrenoceptors.

SHR were also studied for effects of postural changes on MAP after intermittent 90° head-up tilt. For fiduxosin, doses of 0.3 and 1  $\mu\text{mol/kg}$  i.v. (178–592  $\mu\text{g/kg}$  i.v.) failed to lower blood pressure markedly, and there was no further diminution upon head-up tilt (Fig. 6). Fiduxosin (3  $\mu\text{mol/kg}$  or 1780  $\mu\text{g/kg}$  i.v.) slightly reduced MAP, but head-up tilt caused further diminution of MAP at only the 15-min observation with minimal additional changes in MAP at times  $\geq 30$  min postdosing (Fig. 6). At the highest dose of fiduxosin (10  $\mu\text{mol/kg}$  or 5920  $\mu\text{g/kg}$  i.v.), a moderate additive effect to the postural hypotensive response was noted beyond 30 min after compound administration (Fig. 6). However, these hypotensive responses to tilt were considerably less marked than

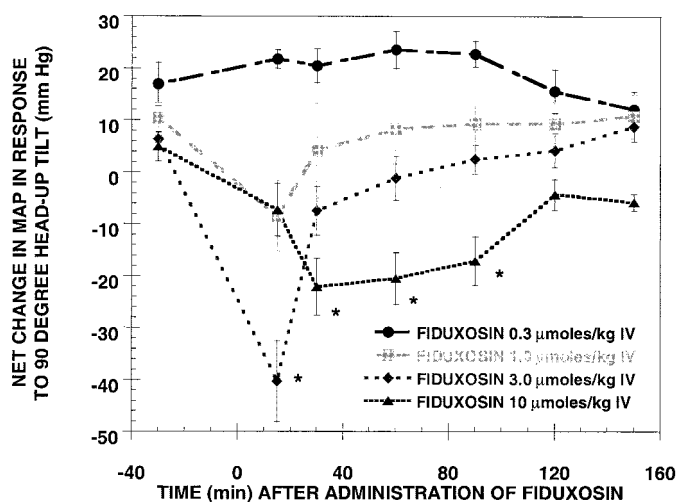
those observed with other  $\alpha_1$ -antagonist compounds (Hancock et al., 1998b).

## Discussion

Pharmacotherapy has become the treatment of choice for new cases of BPH with more than 80% of patients of primary care physicians being prescribed an  $\alpha_1$ -antagonist (Narayan and Tewari, 1998). Successful amelioration of symptoms has been observed with each of the long-acting  $\alpha_1$ -blockers currently approved for BPH, terazosin, doxazosin, and tamsulosin (Narayan and Tewari, 1998). Nevertheless, adverse events often limit effective pharmacotherapy (de Mey, 2000). Postural hypotension or other cardiovascular side effects may be related to the relative lack of  $\alpha_1$ -adrenoceptor subtype selectivity of agents such as terazosin and doxazosin as shown by the lower incidence of these events with tamsulosin (Take et al., 1998), a compound with high potency for  $\alpha_{1a}$ -adrenoceptors, albeit modest subtype selectivity (Hancock, 1996). A number of compounds highly selective for the  $\alpha_{1a}$ -adrenoceptor have been identified in recent years (Testa et al., 1994; Forray et al., 1995; Wetzel et al., 1995; Ford et al., 1996) in search of a uroselective agent for lower urinary tract symptoms associated with BPH, but to date, none have passed clinical development hurdles to validate the proposition that  $\alpha_{1a}$ -adrenoceptor selectivity can enhance the efficacy or reduce the side effect incidence more successfully than currently available agents. Fiduxosin represents a new class of  $\alpha_1$ -antagonists that may offer the potential to treat BPH based on its selective blockade of prostatic or lower urinary tract  $\alpha_1$ -adrenoceptors compared with its effects on cardiovascular responses.

Fiduxosin demonstrated high affinity for  $\alpha_{1a}$ -adrenoceptors in radioligand binding studies and  $\alpha_{1a}$ -adrenoceptors in functional bioassays, and a high degree of selectivity for these sites in comparison with  $\alpha_{1b}$ - or  $\alpha_{1d}$ -adrenoceptors. In radioligand binding assays, the compound was 155-fold selective for  $\alpha_{1a}$ -adrenoceptors, whereas tamsulosin was only 21-fold  $\alpha_{1a}$ - versus  $\alpha_{1b}$ -selective. If the lower incidence of cardiovascular side effect liability encountered with tamsulosin results from this 21-fold selectivity then fiduxosin would be expected to have an even lower incidence of cardiovascular effects. This would contrast with the clinical profile of nonselective  $\alpha_1$ -blockers such as, e.g., terazosin and doxazosin, which generally are slightly more potent antagonists of the  $\alpha_{1b}$ -adrenoceptor (Hancock, 1996). However, the clinical profile of tamsulosin may be a function of both receptor selectivity and optimization of formulation used in therapy of BPH, or perhaps higher prostatic concentrations of the drug, compared with plasma, at least in the dog (Sato et al., 2001). Thus, the clinical inadequacy of REC 15/2739 or Ro-70-0004 might have resulted from pharmacodynamic components and/or their lack of antagonistic action on  $\alpha_{1d}$ -adrenoceptors. In contrast, key pharmacological (selective blockade of both  $\alpha_{1a}$ - and  $\alpha_{1d}$ -adrenoceptors) and pharmacokinetic properties of fiduxosin (long half-life and prolonged in vivo efficacy; Witte et al., 2002) may contribute to a more favorable clinical profile.

Fiduxosin has similar affinities at  $\alpha_{1a}$ - and  $\alpha_{1d}$ -adrenoceptors in radioligand binding (differences of 6-fold or less), whereas tamsulosin was only 2-fold less potent at  $\alpha_{1d}$ - compared with  $\alpha_{1a}$ -adrenoceptors. These selectivity profiles are



**Fig. 6.** Effects of fiduxosin on head-up tilt responses in conscious SHR. Male SHR as described in Fig. 5 were subjected to periodic head-up tilt as described under *Experimental Procedures* and the effects of fiduxosin on tilt-induced hypotensive activity were compared using paired *t* tests, comparing pre- and post-tilt responses at each time point after compound administration. Asterisks indicate data where statistically significant effects of tilt on blood pressure were observed.

generally maintained in the isolated tissue studies where fiduxosin was 5-fold more potent at  $\alpha_{1A}$ -adrenoceptors in rat vas deferens compared with its potency at  $\alpha_{1D}$ -adrenoceptors in the rat aorta, whereas tamsulosin actually showed higher potency at rat aortic  $\alpha_{1D}$ -adrenoceptors than in any other tissue studied. Although the exact role of  $\alpha_{1D}$ -adrenoceptors remains enigmatic, these receptors may have a role in bladder function (Broten et al., 1998; Malloy et al., 1998), such that antagonism of these sites could reduce bladder-related dysfunctional components of lower urinary tract symptoms in BPH (Schwinn and Michelotti, 2000). In addition, antagonism of  $\alpha_{1D}$ -adrenoceptors in the central sympathetic innervation of the prostate and bladder by  $\alpha_{1A}$ -/ $\alpha_{1D}$ -selective antagonists may ameliorate irritative symptoms (Smith et al., 1999; Michel et al., 2000).

Despite the selective antagonism of  $\alpha_{1A}$ -adrenoceptors demonstrated by a number of compounds and the predominance of these receptors in prostatic smooth muscle both from a functional and molecular biological perspective, the failure of several  $\alpha_{1A}$ -adrenoceptor-selective antagonists to demonstrate blockade of both obstructive and irritative symptoms has led to uncertainty regarding the role of  $\alpha_{1A}$ -adrenoceptors in BPH symptomatology. In addition, some studies have indicated pharmacological heterogeneity of prostatic  $\alpha_1$ -adrenoceptors (Muramatsu et al., 1994). Several investigators (Muramatsu et al., 1994; Leonardi et al., 1997; Martin et al., 1997; Testa et al., 1997) have proposed that other receptor interactions might be of functional importance in controlling prostatic tone and contribute to uroselectivity. Prostatic  $\alpha_1$ -adrenoceptors have been suggested to belong to the  $\alpha_{1L}$ -class of adrenoceptors because of their low affinity for prazosin (Muramatsu et al., 1994), and the pharmacological effects of REC 15/2739 have been linked to blockade of  $\alpha_{1L}$ -receptors (Leonardi et al., 1997; Testa et al., 1997). In vitro models of the  $\alpha_{1L}$ -adrenoceptor have been proposed (Muramatsu et al., 1995), including rabbit urethral tissue (Leonardi et al., 1997; Testa et al., 1997), and it has been suggested that blockade of these receptors would be important to the amelioration of BPH (Leonardi et al., 1997; Testa et al., 1997). Our studies with fiduxosin demonstrated antagonism of contractions mediated by urethral  $\alpha_1$ -adrenoceptors, although the potency of fiduxosin to block these sites was considerably weaker than the potency observed at  $\alpha_{1A}$ -adrenoceptors. However, the rank order of potencies of a number of selective and nonselective  $\alpha_1$ -antagonists correlated best with the potency order at  $\alpha_{1a}$ -adrenoceptors, less well with  $\alpha_{1d}$ -adrenoceptors, and very poorly with  $\alpha_{1b}$ -adrenoceptors. Thus, there appeared to be a frame shift in the observed potencies of compounds at urethral  $\alpha_{1L}$ - compared with  $\alpha_{1A}$ -adrenoceptors. These results, and the observations that potency at  $\alpha_{1a/A}$ -adrenoceptors can be modulated by assay conditions (Ford et al., 1997), are consistent with the concept that urethral  $\alpha_1$ -adrenoceptors belong to the  $\alpha_{1A}$ -class. Irrespective of the nomenclature used, the present data clearly indicate that fiduxosin can antagonize urethral  $\alpha_1$ -adrenoceptors more potently than  $\alpha_{1B}$ -adrenoceptors, suggestive of uroselectivity. In contrast, terazosin and tamsulosin, both efficacious in BPH, are more potent at  $\alpha_{1B}$ -adrenoceptors in vitro than at rabbit urethral  $\alpha_1$ -adrenoceptors. In addition, fiduxosin is approximately 10-fold more potent at urethral  $\alpha_1$ -adrenoceptors than terazosin, suggesting that a com-

pound exhibiting this enhanced potency would offer effective blockade of these sites in the amelioration of BPH symptoms.

The selectivity profile obtained with fiduxosin also suggests that the compound would have lower cardiovascular effects than other non- or weakly selective agents such as terazosin, doxazosin, or tamsulosin. This hypothesis was supported by results obtained in SHR, in which fiduxosin was less hypotensive than other agents on a mole per kilogram basis, and also elicited weaker, more transient effects in a postural hypotension challenge. Similar results have been reported with the  $\alpha_{1a}$ -selective compounds Ro-70-0004 (Williams et al., 1999), RWJ-38063, and RWJ-69736 (Pulito et al., 2000), supporting the importance of  $\alpha_{1b}$ -adrenoceptors for cardiovascular function as suggested by data with knockout mice lacking the  $\alpha_{1b}$ -adrenoceptor gene (Cavalli et al., 1997). Recently, a novel compound has been described, B8805-033 (Eltze et al., 2001), with chemical and pharmacological similarities to 5-methyl-urapidil and flesinoxan. B8805-033 is of lower absolute affinity for  $\alpha_{1a}$ -adrenoceptors than fiduxosin but is apparently more selective compared with  $\alpha_{1b}$ - and  $\alpha_{1d}$ -adrenoceptors (150–1200-fold). Interestingly, although also less potent at putative  $\alpha_{1L}$ -sites compared with  $\alpha_{1a}$ -adrenoceptors by approximately 10-fold, B8805-033 maintains high selectivity for  $\alpha_{1L}$ -sites compared with either  $\alpha_{1b}$ - or  $\alpha_{1d}$ -adrenoceptors. However, B8805-033 has higher radioligand binding affinity at 5HT1A sites than at  $\alpha_{1a}$ -adrenoceptors, unlike fiduxosin, which is 180-fold less potent at rat cortical 5HT1A sites. Despite high-affinity agonist activity at 5HT1A receptors comparable in potency to flesinoxan and 5-methyl-urapidil, B8805-033 elicits minimal hypotensive effects in SHR (Eltze et al., 2001). B8805-033 shows properties of uroselectivity in the anesthetized dog, with a selectivity ratio of approximately 52. Although the uroselectivity indices differ between studies of B8805-033 and our protocol with fiduxosin, both data sets suggest that compounds with low affinity for  $\alpha_{1b}$ -adrenoceptors (such as B8805-033 or fiduxosin) compared with  $\alpha_{1a}$ -,  $\alpha_{1L}$ - or  $\alpha_{1d}$ -adrenoceptors may have uroselective properties. Whether the preclinical models available can also elucidate the relative contribution of  $\alpha_{1d}$ -adrenoceptor blockade to amelioration of clinical symptoms of BPH may require clinical evaluation. However, it is of interest to note that although fiduxosin has a high affinity for  $\alpha_{1d}$ -adrenoceptors, the compound elicits minor cardiovascular effects. This would suggest that the  $\alpha_{1d}$ -adrenoceptor does not play a substantial role in blood pressure control. This is underscored by the lower correlation between  $\alpha_{1d}$ -adrenoceptor potency and hypotension (correlation coefficient of 0.63; Fig. 4F) compared with  $\alpha_{1b}$ -adrenoceptor potency (correlation coefficient of 0.94; Fig. 4E). In contrast, a similar analysis with a battery of less subtype-selective compounds was unable to distinguish a substantial difference in the potential contributions of the  $\alpha_{1b}$ - and  $\alpha_{1d}$ -adrenoceptor subtypes. In that previous analysis (Table 9 of Hancock, 1996), the correlations between  $\alpha_{1b}$ - and  $\alpha_{1d}$ -adrenoceptor potency and hypotensive changes in SHR ranged from 0.77 to 0.91. However, the addition of more selective compounds such as fiduxosin to this analysis would suggest that  $\alpha_{1d}$ -adrenoceptors are less important for blood pressure control than  $\alpha_{1b}$ -adrenoceptors.

In contrast to weak cardiovascular effects with fiduxosin, the compound elicited highly potent antagonism of prostatic contractile responses to epinephrine in anesthetized dogs,

confirming the *in vivo* efficacy of fiduxosin. In addition, the ratio between IUP effects in dogs and hypotensive effects in SHR was consistent with the high selectivity for prostatic effects seen with other  $\alpha_{1A/D}$ -selective compounds (Hancock et al., 1998a,b) and indicative that fiduxosin would also selectively antagonize prostatic versus cardiovascular  $\alpha_1$ -adrenoceptors *in vivo*. The results of additional studies that highlight selective blockade of prostatic compared with cardiovascular  $\alpha_1$ -adrenoceptor-mediated effects in conscious dogs will be presented subsequently (Brune et al., 2002). In summary, preferential antagonism of fiduxosin for  $\alpha_{1A}$ - and  $\alpha_{1D}$ - versus  $\alpha_{1B}$ -adrenoceptors *in vitro*, the blockade of putative  $\alpha_{1L}$ -sites, and selective effects on lower urinary tract function versus blood pressure *in vivo* suggest the potential utility of this compound for the treatment of BPH.

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