1521-0103/371/1/106–112$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

https://doi.org/10.1124/jpet.119.260216
J Pharmacol Exp Ther 371:106–112, October 2019

The Journal of PHARMACOLOGY And Experimental Therapeutics

Revisiting the Pharmacodynamic Uroselectivity of α_1-Adrenergic Receptor Antagonists[S]

Bruna Maria Castro Salomão Quaresma, Amanda Reis Pimenta, Anne Caroline Santos da Silva, André Sampaio Pupo, Luiz Antonio S. Romeiro, Claudia Lucia Martins Silva, and François Noël

Laboratory of Biochemical and Molecular Pharmacology, Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (B.M.C.S.Q., A.R.P., A.C.S.d.S., C.L.M.S., F.N.); Department of Pharmacology, Instituto de Biociências, UNESP, Botucatu, Brazil (A.S.P.); and Health Sciences Faculty, Universidade de Brasília, Brasília, Brazil (L.A.S.R.)

Received May 21, 2019; accepted July 2, 2019

ABSTRACT

α_1-Adrenoceptor (AR) antagonists are widely used for the relief of urinary retention secondary to benign prostatic hyperplasia (BPH). While the five Food and Drug Administration–approved α_1-AR antagonists (terazosin, doxazosin, alfuzosin, tamsulosin, and silodosin) share similar efficacy, they differ in tolerability, with reports of ejaculatory dysfunction. The aim of the present work was to revisit their α_1-AR subtype selectivity as well as of LDT5 (1-(2-methoxyphenyl)-4-[2-(3,4-dimethoxyphenyl) ethyl]piperazine monohydrochloride), a compound previously described as a multitarget antagonist of α_{1A}-/α_{1D}-AR and 5-HT_{1A} receptors, and to estimate their affinity for D_2, D_3, and 5-HT_{1A} receptors, which are putatively involved in ejaculatory dysfunction. Competition binding assays were performed with native (D_2, 5-HT_{1A}) or transfected (human α_{1A}-, α_{1B}-, α_{1Dt}-AR, and D_3) receptors for determination of the drug's affinities. Tamsulosin and silodosin have the highest affinities for α_{1A}-AR, but only silodosin is clearly a selective α_{1A}-AR antagonist, with K_i ratios of 25.3 and 50.2 for the α_{1D}- and α_{1B}-AR, respectively. Tamsulosin, silodosin, and LDT5 (but not terazosin, doxazosin, and alfuzosin) have high affinity for the 5-HT_{1A} receptor (K_i around 5–10 nM), behaving as antagonists. We conclude that the uroselectivity of tamsulosin is not explained by its too-low selectivity for the α_{1A}- versus α_{1B}-AR, and that its affinity for D_2 and D_3 receptors is probably too low for explaining the ejaculatory dysfunction reported for this drug. Present data also support the design of "better-than-LDT5" new multitarget lead compounds with pharmacokinetic selectivity based on poor brain penetration and that could prevent hyperplastic cell proliferation and BPH progression.

SIGNIFICANCE STATEMENT

The present work revisits the uroselectivity of the five Food and Drug Administration–approved α_1 adrenoceptor antagonists for the treatment of benign prostatic hyperplasia (BPH). Contrary to what has been claimed by some, our results indicate that the uroselectivity of tamsulosin is probably not fully explained by its too-weak selectivity for the α_{1A} versus α_{1B} adrenoceptors. We also show that tamsulosin affinity for D_3 and 5-HT_{1A} receptors is probably too low for explaining the ejaculatory dysfunction reported for this drug. Based on our lead compound LDT5, present data support the search for a multitarget antagonist of α_{1A}–α_{1D} and 5-HT_{1A} receptors with poor brain penetration as an alternative for BPH treatment.

Introduction

Benign prostatic hyperplasia (BPH) is an age-related disease affecting the quality of life of men mainly due to bladder outlet obstruction, among other bothersome lower urinary tract symptoms (LUTS), such as urgency and nocturia (Berry et al., 1984). α_{1A}-Adrenoceptor (AR) and α_{1D}-AR mRNA have been described in normal and hyperplastic stromal human prostates, and the expression of α_{1A}-ARs is upregulated during BPH (Price et al., 1993; Faure et al., 1994; Nasu et al., 1996; Walden et al., 1999; Roehrborn and Schwinn, 2004; Kojima et al., 2006). The stromal α_{1A}-ARs have been considered important for human prostate contraction (Forray et al., 1994) and, consequently, for the dynamic component of BPH, so that their blockade would explain the observed relief of the micturition difficulties observed with antagonists. On the other hand, cellular proliferation in the periurethral region is related to the static component of BPH and is classically treated at advanced stages of the disease (larger prostates) with the association of α_1-AR antagonists (AARAs) and 5-α-reductase inhibitors (Alawamlh et al., 2018). However,

This study was supported by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil. B.M.C.S.Q. was recipient of a CAPES Ph.D. fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes, Brazil). A.R.P. and A.C.S.d.S. thank CNPq for the scholarships. A.R.P., C.L.M.S., and F.N. are supported by CNPq.
https://doi.org/10.1124/jpet.119.260216.
[S] This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: AARA, α_1-AR antagonist; AR, α_1-adrenoceptor; BPH, benign prostatic hyperplasia; FDA, Food and Drug Administration; LUTS, lower urinary tract symptoms; p-MPPF, 4-Fluoro-N-(2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl)-N-(2-pyridinyl)benzamide.

other receptors are now being considered putative targets for blocking cellular proliferation, such as the α_{1D}-ARs and 5-HT_{1A} receptors (McVary et al., 2011; Oelke et al., 2013). 5-HT_{1A} receptors are considered as an attractive target for antiproliferative drugs since 5-HT acts as a growth factor on several types of nontumoral and tumoral cells (Fiorino et al., 2014). Earlier works already reported that neuroendocrine cells are present in normal and malignant prostate tissue releasing 5-HT (Abrahamsson et al., 1986), and that prostate cells, including those from BPH patients, express 5-HT_{1A} receptors (Dizeyi et al., 2004). Moreover, these authors showed that prostate cell proliferation was reduced by NAN190, a 5-HT_{1A} receptor antagonist (Dizeyi et al., 2004). Finally, we previously showed that LDT5 inhibited the in vitro growth of prostate cells from BPH patients, induced by 5-HT, similarly to that observed for p-MPPF, a classic 5-HT_{1A} receptor antagonist (Nascimento-Viana et al., 2016). Based on these data, we proposed that a multitarget antagonist toward the α_{1A}-AR, α_{1D}-AR, and 5-HT_{1A} receptor, such as LDT5 (Nascimento-Viana et al., 2016), could be a rational nonhormonal alternative in the search of new drugs for the pharmacotherapy of BPH.

Moderate to severe LUTS associated with BPH are mainly treated with AARAs. The five Food and Drug Administration (FDA)–approved AARAs for BPH treatment have similar efficacies, but they differ in tolerability (Schwinn and Roehrborn, 2008; Michel, 2010; Oelke et al., 2013). The so-called uroselective drugs (tamsulosin, silodosin, and alfuzosin) are better tolerated and have a lower incidence of orthostatic hypotension than the first-generation drugs (terazosin and doxazosin) (Michel, 2010; Hennenberg et al., 2014). As commented by Korstanje et al. (2011), uroselectivity has been classically defined in terms of α_1-AR subtype selectivity (pharmacological uroselectivity), preferential reduction of urethral pressure versus blood pressure in animals (functional/physiologic uroselectivity), or desired clinical effects on obstruction and LUTS versus unwanted adverse effects (clinical uroselectivity). As differences exist between the K_i values and selectivities for the five FDA-approved AARA among laboratories (Supplemental Material; Table 1), claims such as tamsulosin's selectivity for α_{1A}-AR should be carefully checked.

Here, we compared these five drugs in the exact same experimental conditions with respect to their affinities for the three human α_1-AR subtypes, together with our LDT5 compound. Furthermore, we considered their selectivity not only toward the classic off-target α_{1B}-AR but also toward the D_3 and 5-HT_{1A} receptors, putatively responsible for sexual disorders, such as abnormal ejaculation, reported for silodosin and tamsulosin (Giuliano et al., 2006; Wolters and Hellstrom, 2006; Andersson and Abdel-Hamid, 2011; Lepor et al., 2012; La Torre et al., 2016).

Different from silodosin and contrary to what has been claimed by some, our results indicate that the uroselectivity of tamsulosin is probably not fully explained by its too-weak α_{1A}- versus α_{1B}-AR selectivity. We also showed that tamsulosin affinity for D_3 and 5-HT_{1A} receptors is probably too low for explaining the ejaculatory dysfunction reported for this drug. Finally, we discuss how multitarget antagonists of α_{1A}–α_{1D} and 5-HT_{1A} receptors, such as LDT5, could be planned for avoiding safety problems at the central nervous system.

Materials and Methods

HEK-293 Cells Transfected with Human α_1-AR

Human embryonic kidney (HEK-293; CRL-1573; American Type Culture Collection) was transfected with human α_{1A}-AR, α_{1B}-AR, and α_{1Dt}-AR (Pupo et al., 2003). As the recombinant full-length human α_{1D}-AR is poorly expressed in recombinant systems, a truncated mutant in which the first 79 amino acids were deleted (α_{1Dt}-AR) was used to increase the number of binding sites (Pupo et al., 2003; Nojimoto et al., 2010) The cells were cultured in Dulbecco's modified Eagle's medium (GIBCO) containing 25 mM glucose, 44 mM sodium bicarbonate, 10% fetal bovine serum from South America, 1% pyruvate, and 1% penicillin (10,000 U/ml)/streptomycin (10,000 μg/ml; Invitrogen) and incubated (37°C, 5% CO_2) until confluence when they were washed with 1 ml of phosphate-buffered saline and scraped to obtain the homogenate. Subsequently, the homogenate was centrifuged at 30,000*g* for 20 minutes at 4°C, the supernatant discarded, and the pellet resuspended in approximately 10 ml of solution (25 mM HEPES, 150 mM NaCl, 3 mM phenylmethylsulfonyl fluoride, and 1 mM protease inhibitor cocktail, pH 7.4). This material was homogenized with an Ultra-Turrax (IKA Labortechnik) apparatus (twice for 15 seconds at a speed of 9500 rpm). The homogenate was then centrifuged at 30,000*g* for 20 minutes at 4°C, the supernatant was discarded, and the new pellet was resuspended in buffer containing 25 mM HEPES and 150 mM NaCl, pH 7.4 (Akinaga et al., 2013).

Binding Experiments

Binding to the α_1-ARs. The membrane preparation of transfected HEK-293 cells (150 μg of protein) was incubated for 45 minutes at 30°C in 1 ml of medium containing 0.05 nM [^{3}H]-prazosin, 50 mM Tris-HCl 50 mM (pH 7.4), and 1 mM EDTA

TABLE 1
Affinity (K_i values) and selectivity (ratios of K_i values) for binding of α_1-AR antagonists to the three human α_1-AR subtypes
K_i values are expressed as geometric means of [*n*] individual experiments. One-way ANOVA and post hoc Holm-Sidak test was performed on pK_i values.

Compounds	K_i (95% CI) α_{1A} (1)	K_i (95% CI) α_{1Dt} (2)	K_i (95% CI) α_{1B} (3)	K_i Ratio (2)/(1)	K_i Ratio (3)/(1)
	nM	*nM*	*nM*		
LDT5	3.82[a] [3] (1.57–9.29)	4.94[a] [3] (19.0–9.24)	9.86 [3] (6.77–14.4)	1.29	2.90
Tamsulosin	0.36 [3] (0.11–1.17)	1.05[b] [3] (0.37–2.91)	1.85[c] [3] (1.36–5.50)	2.92	5.10
Silodosin	0.44 [3] (0.22–0.85)	11.1[d] [3] (5.17–23.8)	22.1[d] [3] (9.37–52.2)	25.2	50.2
Alfuzosin	11.4[e] [4] (4.31–30.4)	4.83 [4] (2.56–9.12)	2.35 [4] (1.18–4.68)	0.42	0.20
Terazosin	11.2[a] [4] (7.75–16.1)	6.67 [4] (2.61–17.0)	3.63 [4] (1.83–7.23)	0.59	0.32
Doxazosin	3.60 [4] (1.16–11.1	1.58 [4] (0.99–2.53)	2.16 [4] (1.40–3.32)	0.44	0.60

CI, confidence interval.
[a] $P < 0.05$ vs. AR-α_{1B}.
[b] $P < 0.05$ vs. AR-α_{1A}.
[c] $P < 0.01$ vs. AR-α_{1A}.
[d] $P < 0.0001$ vs. AR-α_{1A}.
[e] $P < 0.01$ vs. AR-α_{1B}.

(Nascimento-Viana et al., 2016). Nonspecific binding was defined in the presence of 1 μM prazosin. The incubation was terminated by filtration, washing, and treatment of the filters as described previously (Nascimento-Viana et al., 2016).

Binding to the 5-HT$_{1A}$ Receptor. For binding assays to the 5-HT$_{1A}$ receptors, hippocampi of adult male Wistar rats were homogenized and centrifuged as previously described (Noël et al., 2014). Binding to the low-affinity and high-affinity states of the receptor was performed as detailed previously together with the rationale for estimating the intrinsic efficacy of the ligands by using the K_i ratio (Noël et al., 2014). The protein was incubated at 37°C under yellow light for either 45 minutes with 0.5 nM [^{3}H]-p-MPPF (4-Fluoro-N-(2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl)-N-(2-pyridinyl)benzamide), 50 mM Tris-HCl (pH 7.4), and 1 mM GTP (low-affinity state) or 15 minutes in a solution containing 1 nM [^{3}H]-8-OH-DPAT, 1 mM $CaCl_2$, 1 mM $MnCl_2$, 10 mM pargyline, and Tris-HCl 50 mM (pH 7.4; high-affinity state).

Binding to the D$_2$ and D$_3$ Receptors. For binding assays to the D$_2$-like receptors, the striatum of adult male Wistar rats was homogenized and centrifuged as previously described (Pompeu et al., 2013; protocol number DFBCICB021, Institutional Ethical Committee for Animal Care from the Federal University of Rio de Janeiro). For binding to the D$_3$ receptor, we used commercially available (Chemiscreen; Millipore) crude membrane preparations of recombinant Chem-1 cells that have been transfected with the cDNA encoding the human D$_3$ receptor (accession number NM_000796). Membranes, compounds, and radioligand (0.1 nM [^{3}H]-YM-09151-2) were incubated at 37°C for 60 minutes under yellow light in a solution containing 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 1 mM EDTA, and Tris-HCl 50 mM (pH 7.4) as previously described (Betti et al., 2017).

Statistical Analysis

Data were analyzed by nonlinear regression using GraphPad Prism 6.0 (GraphPad Software) using the classic equations for simple concentration-effect curves (saturation experiments) and competition binding assays to estimate affinity (K_d) of the radioligand and potency [median inhibitory concentrations (IC_{50})] of the unlabeled competitor ligands, respectively. The affinity of the unlabeled competitor ligands (K_i) was calculated using the IC_{50} values and the Cheng-Prusoff equation (Cheng and Prusoff, 1973). K_i values were expressed as geometric means with their 95% confidence interval.

Drugs

[^{3}H]-Prazosin (85 Ci/mmol), [^{3}H]-8-OH-DPAT (154.2 Ci/mmol), [^{3}H]-p-MPPF (74.2 Ci/mmol), and [^{3}H]-YM-09151-2 (81.1 Ci/mmol) were purchased from PerkinElmer. Alfuzosin hydrochloride, doxazosin mesylate, tamsulosin hydrochloride, and terazosin hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO), and silodosin was from ShangHai Biochempartner (China). LDT5 hydrochloride ((1-(2-methoxyphenyl)-4-[2-(3,4-dimethoxyphenyl) ethyl] piperazine monohydrochloride) was synthesized as previously described for other *N*-phenylpiperazine derivatives (Romeiro et al., 2011). Stock solutions (1 and 10 mM) were made in sterile deionized water (LDT5) or 100% DMSO (Sigma-Aldrich) and then diluted in water. At the final concentration used (no more than 0.1%), DMSO had no effect in our assays.

Results

Determination of K_d for [^{3}H]-Prazosin Binding to the Three Human α_1-ARs. We first characterized the binding of [^{3}H]-prazosin to the three subtypes of human α_1-AR by performing saturation experiments at equilibrium to determine the K_d values in our experimental conditions. The K_d values were 0.475, 0.354, and 0.577 nM for the α_{1A}-, α_{1B}-, and α_{1Dt}-ARs, respectively (geometric means, $n = 2$), and were similar to values described elsewhere with these cells (Nojimoto et al., 2010).

Determination of K_i Values and Selectivity of Test Compounds for Binding at the Human α_1-ARs. As illustrated in Fig. 1, we performed full competition curves for the three human α_1-AR subtypes with our six compounds using the antagonist [^{3}H]-prazosin as the radioligand. Note that the potency sequence is somewhat different for these three subtypes as exemplified by silodosin, one of the most potent for inhibiting [^{3}H]-prazosin binding to the α_{1A}-AR

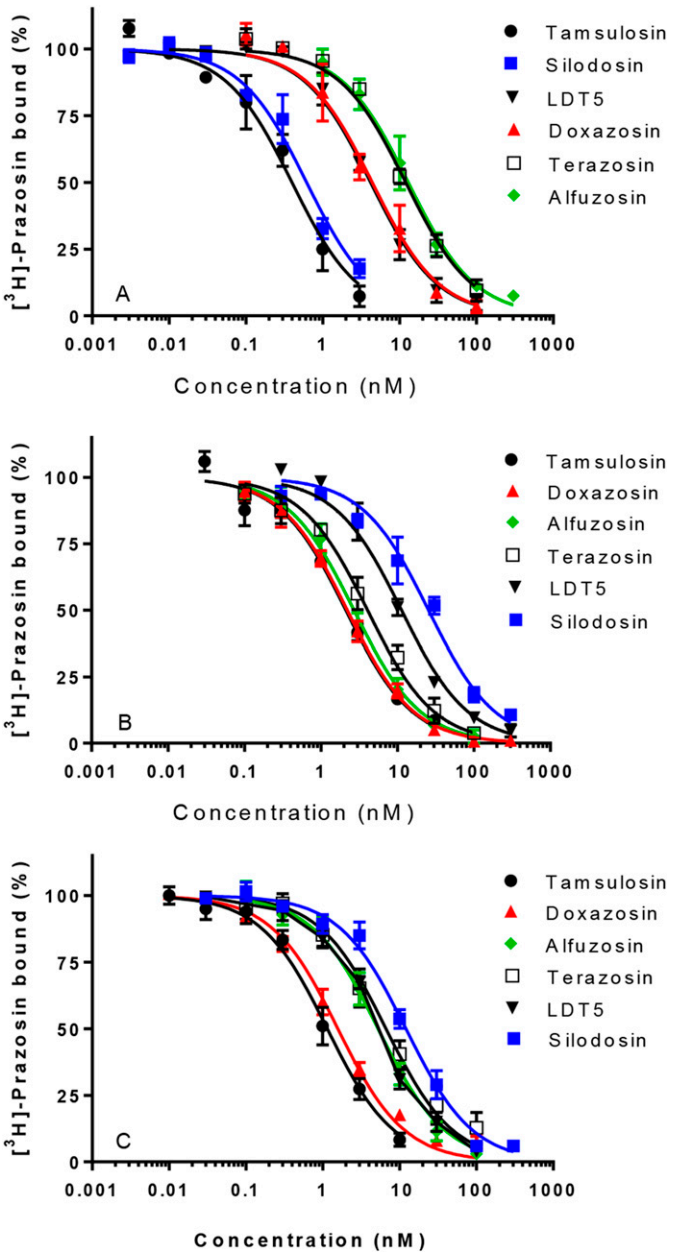


Fig. 1. Effect of α_1-AR antagonists on the binding of [^{3}H]-prazosin to human α_{1A}-AR (A), α_{1B}-AR (B), and α_{1D}-AR (C). The membrane preparation of transfected HEK-293 cells (150 μg of protein) was incubated for 45 minutes at 30°C in 1 ml of medium containing 0.05 nM [^{3}H]-prazosin, Tris-HCl 50 mM (pH 7.4), and 1 mM EDTA in the presence or absence of increasing concentrations of the tested compounds. The data represent the mean ± S.E.M. of three to four independent experiments performed in triplicate.

(Fig. 1A) but less potent for the α_{1B}-AR (Fig. 1B) and α_{1D}-AR (Fig. 1C).

Tamsulosin and silodosin have the highest affinities for the α_{1A}-AR but differ mainly with respect to their selectivity profile: whereas tamsulosin affinity for α_{1A}-AR is only slightly higher than for α_{1D}- and α_{1B}-AR, with K_i ratios of 2.92 and 5.1, respectively, silodosin is clearly an α_{1A}-AR selective ligand with K_i ratios of 25.3 and 50.2 for the α_{1D}- and α_{1B}-AR, respectively (Supplemental Fig. 1; Table 1).

With respect to the third uroselective drug, alfuzosin has an affinity 2–4 times lower for the α_{1A}-AR than for the other two subtypes, as also observed with terazosin. Alfuzosin and terazosin showed similar affinities for α_{1A}-AR and similar selectivity profiles (Supplemental Fig. 1; Table 1).

Doxazosin has a similar affinity for the three subtypes. Our compound LDT5 has the same affinity for the α_{1A}- and α_{1D}-ARs, being around 2 to 3 times higher than that for α_{1B}-AR, showing a selectivity profile similar to tamsulosin (Supplemental Fig. 1; Table 1).

Determination of Affinity and Intrinsic Efficacy of Test Compounds at the 5-HT$_{1A}$ Receptor. To determine the affinity of the six compounds to the 5-HT$_{1A}$ receptor, we used a binding assay with the antagonist radioligand [^{3}H]-p-MPPF and rat hippocampal membranes, as previously described (Noël et al., 2014).

Table 2 shows that LDT5, tamsulosin, and silodosin have a high affinity for this receptor, with K_i values around 5–10 nM, whereas alfuzosin, terazosin, and doxazosin have a much lower affinity, with K_i values higher than 1 μM. Tamsulosin had a K_i value close to the one reported previously by others (4.4 nM; Leonardi et al., 1997). Considering the affinity for the main target receptor of BPH involved in contraction (α_{1A}-AR) as a reference, Table 2 indicates that LDT5 has the same affinity for the 5-HT$_{1A}$ (K_i ratio equal to 1.56) and that silodosin affinity for 5-HT$_{1A}$ is relevant (K_i ratio around 10). On the contrary, albeit tamsulosin binds to 5-HT$_{1A}$ at nanomolar concentrations, its affinity is about 33 times lower than for the α_{1A}-AR. With K_i ratios much higher than 100, alfuzosin, terazosin, and doxazosin are to be considered highly selective α_{1A}-AR ligands toward the 5-HT$_{1A}$ receptor.

Because not only affinity but also intrinsic efficacy is important for pharmacological effect, we then used a previously validated functional binding assay (Noël et al., 2014) for the three compounds with relevant affinity for the 5-HT$_{1A}$ receptor. As described in Fig. 2 for silodosin and tamsulosin, competition curves were performed using either the antagonist radioligand [^{3}H]-p-MPPF in the presence of GTP (low-affinity state of the receptor) or the agonist radioligand [^{3}H]-8-OH-DPAT in the presence of divalent cations that favor the high-affinity state of the receptor. In such an assay, the intrinsic efficacy of a compound is estimated by its ratio of K_i values measured when the receptor is in the low- to high-affinity state. With K_i ratios not different from 1, LDT5 and silodosin are to be considered as neutral antagonists, whereas tamsulosin harbored a K_i ratio of 3.9, significantly different from 1 (Table 2). As this ratio is much smaller than the one reported for the full agonist 5-HT [76.8; see Noël et al. (2014)], tamsulosin is to be considered as a weak partial agonist of this receptor.

Determination of Affinity of Tamsulosin and LDT5 at D$_2$ and D$_3$ Receptors. Due to the putative role of D$_2$ and, mainly, D$_3$ receptors as off targets for drugs used in BPH therapy, we determined the affinity of tamsulosin and LDT5 for human D$_3$ receptors and D$_2$-like receptors present in rat striatal preparations [mainly D$_2$ receptors, according to Booze and Wallace (1995)]. Table 3 shows that both compounds have a higher affinity for the D$_3$ than for the D$_2$ receptors. The K_i ratios (D$_3$ vs. α_{1A}-AR) are around 44 and 8 for tamsulosin and LDT5, respectively.

Discussion

Pharmacological Selectivity of the Five FDA-Approved α_{1A}-AR Antagonists for BPH Treatment. In vitro off-target receptor binding is a well established method of derisking used in drug discovery programs (Bowes et al., 2012) and is also the basis for defining whether the so-called uroselectivity of some α_1-AR antagonists used for BPH treatment is due to pharmacological selectivity or to other reasons (see *Introduction*). Since α_{1B}-ARs are not involved in the pathophysiology of BPH and are expressed in several tissues, including blood vessels, they have been considered for a long time as off target for α_1-AR antagonists used in BPH due to the idea that their blockade was responsible for cardiovascular adverse effects, mainly postural hypotension (Michel, 2010). This idea has now been challenged due to the controversial role of the α_{1B}-AR in controlling blood pressure and the good cardiovascular tolerability of alfuzosin, a nonselective antagonist (Michel, 2010; Akinaga et al., 2019). On the other hand, the α_{1A}-AR is considered the main target for BPH treatment due to the prominence of this subtype in the human prostate and its role

TABLE 2

Affinity (K_i values) for binding to the 5-HT$_{1A}$ receptor (low-affinity state) and selectivity for binding to α_{1A}-AR versus 5-HT$_{1A}$ receptor

K_i values are expressed as geometric means of [n] individual values calculated from competition curves using the antagonist radioligand (low-affinity state, see *Materials and Methods*). The ratio of these K_i values and the K_i values for α_{1A}-AR is a measure of selectivity. Intrinsic activity at the 5-HT$_{1A}$ receptor was estimated by the ratio of K_i values for the low- and high-affinity state of the receptor (Noël et al., 2014).

Compounds	5-HT$_{1A}$: Low K_i [n] (95% CI)	K_i Ratio 5-HT$_{1A}$ Low/α_{1A}-AR	K_i Ratio 5-HT$_{1A}$ (Low/High)
	nM		
LDT5	5.96 [3] (2.64–13.5)	1.56	1.17
Tamsulosin	11.9 [5] (7.96–17.8)	33.0****	3.91$^{\#}$
Silodosin	4.23 [3] (1.21–14.8)	9.61**	1.20
Alfuzosin	2130 [3] (380–11,900)	186***	—
Terazosin	19,890 [3] (2970–133,270)	1776****	—
Doxazosin	4240 [3] (1750–10,270)	1178****	—

CI, confidence interval.

$^{\#}P < 0.01$, unpaired Student's t test on pK_i values (5-HT$_{1A}$ low vs. 5-HT$_{1A}$ high); $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$, unpaired Student's t test on pK_i values (5-HT$_{1A}$ low vs. α_{1A}).

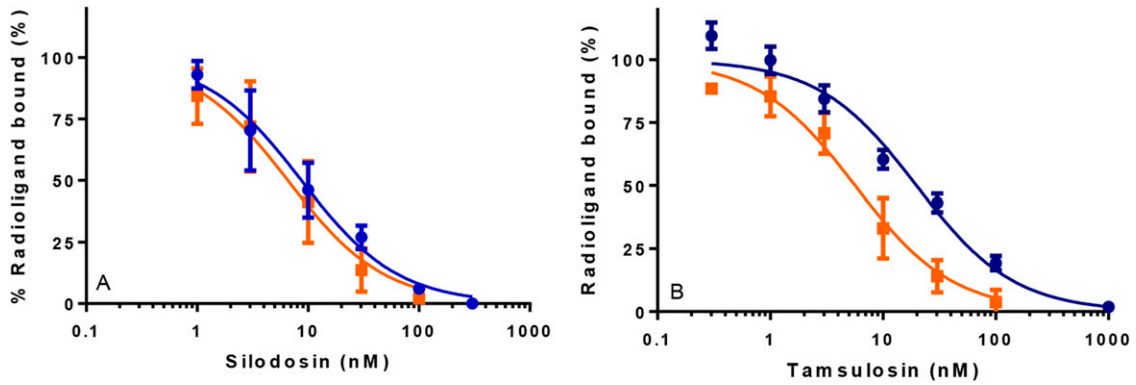


Fig. 2. Specific binding of [^{3}H]-8-OH-DPAT (squares) and [^{3}H]-p-MPPF (circles) in rat hippocampal membranes in the presence of increasing concentrations of silodosin and tamsulosin. The protein was incubated at 37°C for either 45 minutes with 0.5 nM [^{3}H]-p-MPPF, Tris-HCl 50 mM (pH 7.4), and 1 mM GTP (low-affinity state) or 15 minutes in a solution containing 1 nM [^{3}H]-8-OH-DPAT, 1 mM $CaCl_2$, 1 mM $MnCl_2$, 10 mM pargyline, and Tris-HCl 50 mM (pH 7.4; high-affinity state). Data are expressed as means ± S.E.M. of three to five individual experiments performed in triplicate.

in prostate contraction (Forray et al., 1994). As a result of the classic view on BPH, the K_i ratio (α_{1B}-AR/α_{1A}-AR) was used for quantifying pharmacological selectivity of such drugs. As indicated in Table 4, our results support the claimed α_{1A} selectivity of silodosin, since the referred K_i ratio is about 50. On the other hand, our data do not support the usual claim that tamsulosin is an α_{1A}-AR (or $\alpha_{1A/1D}$-AR) selective antagonist. Indeed, our K_i ratio was 5.1, similar to the low values already reported in two other works (Table 4). Note that even the higher K_i ratios reported by two other groups (around 12) are not sufficient to support the claim, as also criticized by Lepor et al. (2012), who considered that no clinical advantage could be attributed to a receptor selectivity of only about 10 times. Based on a 94%–99% binding to plasma proteins (Flomax CR product monograph, tamsulosin, BOEHRINGER INGELHEIM), we can estimate that the free maximal plasma concentration of tamsulosin at steady state after daily administration of a controlled-release tablet containing 0.4 mg of tamsulosin hydrochloride is below or around the K_i we measured for tamsulosin binding to the α_{1B}-AR. As a consequence, a K_i ratio higher than 5 (α_{1B} vs. α_{1A}) would have a clinical relevance since the active (free) plasma concentration would be in the "selective" range. As an alternative, the explanation elegantly proposed by Sato et al. (2012) sounds plausible. These authors reported that the residence time of tamsulosin at the α_{1A}-AR was much higher than that at the α_{1B}-AR subtype, contrary to what occurred with prazosin. Note that a pharmacodynamics selectivity is expected for drugs with a higher residence time at the target than at the off target (Copeland et al., 2006). As an alternative hypothesis to explain tamsulosin's reported uroselectivity, Korstanje et al. (2011) concluded that tamsulosin would exhibit a greater uptake into human prostate than would be expected from plasma concentrations based on differences in unbound drug fraction in human prostate (59%) and plasma (0.4%). Based on these data, the area under the curve (0, 24 hours) of unbound tamsulosin in prostate tissue was estimated to be 63-fold higher than the area under the curve (0, 24 hours) in plasma. As it is assumed that, under equilibrium conditions, diffusion of unbound drug will lead to equal drug concentrations in these two compartments, we cannot discard an experimental artifact since the unbound concentrations were not measured directly through in situ microdialysis, the gold standard approach for such experiments. Our data also confirm that the uroselectivity of alfuzosin is not due to a pharmacological selectivity between the α_1-AR subtypes, since its affinity for the α_{1A}-AR is even slightly lower (higher K_i ratios) than for the two other subtypes, as also observed for the two "old" nonselective α_1-AR antagonists terazosin and doxazosin (Table 4).

In addition to the α_{1B}-AR, we also considered the affinity of the five FDA-approved drugs toward the 5-HT_{1A} and D_2/D_3 receptors, which are poorly discussed in the literature for these drugs. Our interest was based on the proposal that these receptors participate in the central control of ejaculation and could be involved in the ejaculation disorders observed clinically in BPH patients, particularly those treated with silodosin or tamsulosin (Giuliano et al., 2006; Wolters and Hellstrom, 2006; Lepor et al., 2012; La Torre et al., 2016). For silodosin, although harboring some relevant affinity for the 5-HT_{1A} receptor, the central effect could probably be discarded due to its apparently poor brain penetration (Okura et al., 2002). For tamsulosin, the situation is less clear since some penetration into the brain has been reported, albeit without quantitative data (Giuliano et al., 2006), whereas a low potential to cross the blood-brain barrier has also been

TABLE 3
Affinity of tamsulosin and LDT5 for human D_3 and rat striatum
D_2–like receptors and selectivity for binding to the α_{1A}-AR versus D_3 receptor K_i values are expressed as geometric means of [n] individual experiments.

Compounds	K_i (95% CI) D_3	K_i (95% CI) D_2	K_i Ratio D_3/α_{1A}
	nM	*nM*	
Tamsulosin	15.7 [3] (3.7–37.5)	88.9 [4] (73.7–128)	43.6***
LDT5	30.7 [3] (14.7–47.5)	68.7 [5] (57.0–82.8)	8.04***

***$P < 0.001$, t test (pK_i D_3 vs. pK_i α_{1A}).

TABLE 4
Selectivity (ratios of K_i values) for binding to α_1-AR subtypes: between target subtypes and between the main target (α_{1A}) and off-target (α_{1B}) subtypes
Comparison between present data and data from the literature.

Compounds	K_i Ratio α_{1D}/α_{1A}							K_i Ratio α_{1B}/α_{1A}						
	Our Data	1	2	3	4	5	6	Our Data	1	2	3	4	5	6
LDT5	1.29		—	—	—	—	—	2.90		—	—	—	—	—
Tamsulosin	2.92	2.5	3.5	1.00	0.80	—	0.26	5.10	10.0	11.7	12.6	6.3	—	3.30
Silodosin	25.2	56.4	25.6	—	—	—	19.8	50.2	167	25.6	—	—	—	23.2
Alfuzosin	0.42		0.17	0.10	0.32	0.63	—	0.20		0.15	0.13	1.00	0.47	—
Terazosin	0.59		0.52	0.10	—	0.50	0.15	0.32		0.49	0.10	—	0.28	0.05
Doxazosin	0.44		—	1.26	1.26	0.60	—	0.60		—	1.00	0.32	0.38	—

1, Tatemichi et al. (2006); 2, Sato et al. (2012); 3, Richardson et al. (1997); 4, Kenny et al. (1996); 5, Forray et al. (1994) (present nomenclature); 6, Ishiguro et al. (2002).

reported by others (Andersson and Abdel-Hamid, 2011). Our data do not support the participation of the 5-HT_{1A}, D_2, and D_3 receptors in the ejaculation disorders due to a relatively low affinity of tamsulosin for these receptors. Note that the K_i value for the D_3 receptor reported by Kuo et al. (2000) was much lower than ours (0.28 vs. 15.7 nM) and that we did not find any apparent explication for such difference nor other data in the literature.

LDT5 and Insight for Putative New Multitarget Lead Compounds. The present data extend our previous data indicating that LDT5 could be considered a multitarget drug for the $\alpha_{1A/D}$-AR and 5-HT_{1A} receptors (Nascimento-Viana et al., 2016). Previous estimates of affinity (K_B) for the α_{1A}- and α_{1D}-AR were based on the antagonism of phenylephrine-induced isometric contractions of rat prostate and aorta, respectively, whereas affinity for the α_{1B}-AR was assessed by competition for [^{3}H]-prazosin binding to rat liver synaptosomes (K_i). Present affinity estimates were all obtained in binding experiments with membranes of cells transfected with each of the three human α_1-AR subtypes, a priori, a more suitable assay for a translational point of view. Although the affinity for the α_{1A}- and α_{1D} -ARs are lower (8–14 times), the ratio of K_i values for these two receptors is similar, confirming that LDT5 is a high-affinity $\alpha_{1A/D}$-AR ligand. Based on the present data, the selectivity toward the off-target α_{1B}-AR should be lower than previously estimated (2.9 vs. 55 times). However, in vivo LDT5 showed an ED_{50} of 0.09 $\mu g \cdot kg^{-1}$ for the reduction of intraurethral pressure, and a similar dose (0.1 $\mu g \cdot kg^{-1}$) did not cause any hypotensive effect (Nascimento-Viana et al., 2016), which could suggest a potential uroselective profile in rats.

The present data give support for designing "better-than-LDT5" new multitarget ($\alpha_{1A/D}$-AR and 5-HT_{1A} receptor) lead compounds. Indeed, as blockade of brain 5-HT_{1A} receptors could result in on-target adverse effects (see *Pharmacological Selectivity of the Five FDA-Approved α_{1A}-AR Antagonists for BPH Treatment*), a pharmacokinetic selectivity based on poor brain penetration would be a strategy for such compounds, e.g., by designing a drug that would be a substrate of P-glycoprotein. This concern is also strengthened by the relatively high affinity we reported here for LDT5 binding to the D_3 receptor. Considering these data, LDT5 is no more considered as the ideal lead compound since the permeability assay with MDCK-MDR1 showed that it is not a substrate of P-glycoprotein (Noël et al., 2016). However, we suggest that the rationale of such a multitarget drug for BPH treatment is maintained mainly based on our previous data with cells from BPH patients, since LDT5 inhibited prostate hyperplastic cell proliferation and reduced intraurethral pressure without hypotensive effects (Nascimento-Viana et al., 2016).

Authorship Contributions

Participated in research design: Quaresma, Silva, Noël.
Conducted experiments: Quaresma, Pimenta, Santos da Silva.
Contributed new reagents or analytic tools: Romeiro, Pupo.
Performed data analysis: Quaresma, Pimenta, Santos da Silva.
Wrote or contributed to the writing of the manuscript: Quaresma, Pupo, Romeiro, Silva, Noël.

References

Abrahamsson PA, Wadström LB, Alumets J, Falkmer S, and Grimelius L (1986) Peptide-hormone- and serotonin-immunoreactive cells in normal and hyperplastic prostate glands. *Pathol Res Pract* **181**:675–683.
Akinaga J, García-Sáinz JA, and S Pupo A (2019) Updates in the function and regulation of α_1 -adrenoceptors. *Br J Pharmacol* **176**:2343–2357.
Akinaga J, Lima V, Kiguti LR, Hebeler-Barbosa F, Alcántara-Hernández R, García-Sáinz J, and Pupo AS (2013) Differential phosphorylation, desensitization, and internalization of α1A-adrenoceptors activated by norepinephrine and oxymetazoline. *Mol Pharmacol* **83**:870–881.
Alawamlh OAH, Goueli R, and Lee RK (2018) Lower urinary tract symptoms, benign prostatic hyperplasia, and urinary retention. *Med Clin North Am* **102**:301–311.
Andersson KE and Abdel-Hamid IA (2011) Therapeutic targets for premature ejaculation. *Maturitas* **70**:26–33.
Berry SJ, Coffey DS, Walsh PC, and Ewing LL (1984) The development of human benign prostatic hyperplasia with age. *J Urol* **132**:474–479.
Betti AH, Antonio CB, Pompeu TE, Martins TS, Herzfeldt V, Stolz ED, Fraga CA, Barreiro E, Noël F, and Rates SM (2017) LASSBio-1422: a new molecular scaffold with efficacy in animal models of schizophrenia and disorders of attention and cognition. *Behav Pharmacol* **28**:48–62.
Booze RM and Wallace DR (1995) Dopamine D_2 and D_3 receptors in the rat striatum and nucleus accumbens: use of 7-OH-DPAT and [^{125}I]-iodosulpride. *Synapse* **19**: 1–13.
Bowes J, Brown AJ, Hamon J, Jarolimek W, Sridhar A, Waldron G, and Whitebread S (2012) Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. *Nat Rev Drug Discov* **11**:909–922.
Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem Pharmacol* **22**:3099–3108.
Copeland RA, Pompliano DL, and Meek TD (2006) Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov* **5**:730–739.
Dizeyi N, Bjartell A, Nilsson E, Hansson J, Gadaleanu V, Cross N, and Abrahamsson PA (2004) Expression of serotonin receptors and role of serotonin in human prostate cancer tissue and cell lines. *Prostate* **59**:328–336.
Faure C, Pimoule C, Vallancien G, Langer SZ, and Graham D (1994) Identification of alpha 1-adrenoceptor subtypes present in the human prostate. *Life Sci* **54**: 1595–1605.
Fiorino F, Severino B, Magli E, Ciano A, Caliendo G, Santagada V, Frecentese F, and Perissutti E (2014) 5-HT(1A) receptor: an old target as a new attractive tool in drug discovery from central nervous system to cancer. *J Med Chem* **57**:4407–4426.
Forray C, Bard JA, Wetzel JM, Chiu G, Shapiro E, Tang R, Lepor H, Hartig PR, Weinshank RL, Branchek TA, et al. (1994) The α_{1}-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1c} subtype. *Mol Pharmacol* **45**:703–708.
Giuliano FA, Clément P, Denys P, Alexandre L, and Bernabé J (2006) Comparison between tamsulosin and alfuzosin on the expulsion phase of ejaculation in rats. *BJU Int* **98**:876–879.
Hennenberg M, Stief CG, and Gratzke C (2014) Pharmacology of the lower urinary tract. *Indian J Urol* **30**:181–188.
Ishiguro M, Futabayashi Y, Ohnuki T, Ahmed M, Muramatsu I, and Nagatomo T (2002) Identification of binding sites of prazosin, tamsulosin and KMD-3213 with $\alpha_{(1)}$-adrenergic receptor subtypes by molecular modeling. *Life Sci* **71**: 2531–2541.

Kenny BA, Miller AM, Williamson IJR, O'Connell J, Chalmers DH, and Naylor AM (1996) Evaluation of the pharmacological selectivity profile of α_1 adrenoceptor antagonists at prostatic α_1 adrenoceptors: binding, functional and *in vivo* studies. *Br J Pharmacol* **118**:871–878.

Kojima Y, Sasaki S, Shinoura H, Hayashi Y, Tsujimoto G, and Kohri K (2006) Quantification of alpha1-adrenoceptor subtypes by real-time RT-PCR and correlation with age and prostate volume in benign prostatic hyperplasia patients. *Prostate* **66**:761–767.

Korstanje C, Krauwinkel W, and van Doesum-Wolters FLC (2011) Tamsulosin shows a higher unbound drug fraction in human prostate than in plasma: a basis for uroselectivity? *Br J Clin Pharmacol* **72**:218–225.

Kuo GH, Prouty C, Murray W, Pulito V, Jollice L, Cheung P, Varga S, Evangelisto M, and Shaw C (2000) Design, Synthesis and Biological Evaluation of Pyridine-Phenylpiperazines: A Novel Series of Potent and Selective α_{1A}-Adrenergic Receptor Antagonist. *Bioorganic & Medicinal Chemistry* **8**:2263–2275.

La Torre A, Giupponi G, Duffy D, Conca A, Cai T, and Scardigli A (2016) Sexual dysfunction related to drugs: a critical review. Part V: α-blocker and 5-ARI drugs. *Pharmacopsychiatry* **49**:3–13.

Leonardi A, Hieble JP, Guarneri L, Naselsky DP, Poggesi E, Sulpizio AC, and Testa R (1997) Pharmacological Characterization of the Uroselective Alpha-1 Antagonist Rec 15/2739 (SB 216469): Role of the Alpha-1L adrenoceptor in tissue Selectivity. *The Journal of Pharmacology end Experimental Therapeutics* **281** (3):1272–1283.

Lepor H, Kazzazi A, and Djavan B (2012) α-Blockers for benign prostatic hyperplasia: the new era. *Curr Opin Urol* **22**:7–15.

McVary KT, Roehrborn CG, Avins AL, Barry MJ, Bruskewitz RC, Donnell RF, Foster HE Jr, Gonzalez CM, Kaplan SA, Penson DF, et al. (2011) Update on AUA guideline on the management of benign prostatic hyperplasia. *J Urol* **185**:1793–1803.

Michel MC (2010) The forefront for novel therapeutic agents based on the pathophysiology of lower urinary tract dysfunction: alpha-blockers in the treatment of male voiding dysfunction - how do they work and why do they differ in tolerability? *J Pharmacol Sci* **112**:151–157.

Nascimento-Viana JB, Carvalho AR, Nasciutti LE, Alcántara-Hernández R, Chagas-Silva F, Souza PA, Romeiro LA, García-Sáinz JA, Noël F, and Silva CLM (2016) New multi-target antagonists of α_{1A}-, α_{1D}-adrenoceptors and 5-HT_{1A} receptors reduce human hyperplastic prostate cell growth and the increase of intraurethral pressure. *J Pharmacol Exp Ther* **356**:212–222.

Nasu K, Moriyama N, Kawabe K, Tsujimoto G, Murai M, Tanaka T, and Yano J (1996) Quantification and distribution of alpha 1-adrenoceptor subtype mRNAs in human prostate: comparison of benign hypertrophied tissue and non-hypertrophied tissue. *Br J Pharmacol* **119**:797–803.

Noël F, Nascimento-Viana JB, Romeiro LA, Silva RO, Lemes LF, Oliveira AS, Giorno TB, Fernandes PD, and Silva CL (2016) ADME studies and preliminary safety pharmacology of LDT5, a lead compound for the treatment of benign prostatic hyperplasia. *Braz J Med Biol Res* **49**:e5542.

Noël F, Pompeu TE, and Moura BC (2014) Functional binding assays for estimation of the intrinsic efficacy of ligands at the 5-HT_{1A} receptor: application for screening drug candidates. *J Pharmacol Toxicol Methods* **70**:12–18.

Nojimoto FD, Mueller A, Hebeler-Barbosa F, Akinaga J, Lima V, Kiguti LR, and Pupo AS (2010) The tricyclic antidepressants amitriptyline, nortriptyline and imipramine are weak antagonists of human and rat alpha1B-adrenoceptors. *Neuropharmacology* **59**:49–57.

Oelke M, Bachmann A, Descazeaud A, Emberton M, Gravas S, Michel MC, N'dow J, Nordling J, and de la Rosette JJ; European Association of Urology (2013) EAU guidelines on the treatment and follow-up of non-neurogenic male lower urinary tract symptoms including benign prostatic obstruction. *Eur Urol* **64**:118–140.

Okura T, Yamada S, Abe Y, and Kimura R (2002) Selective and sustained occupancy of prostatic a1-adrenoceptors by oral administration of KMD-3213 and its plasma concentration in rats. *Journal of Pharmacy and Pharmacology* **54**:975–982.

Pompeu TET, Alves FRS, Figueiredo CDM, Antonio CB, Herzfeldt V, Moura BC, Rates SMK, Barreiro EJ, Fraga CAM, and Noël F (2013) Synthesis and pharmacological evaluation of new N-phenylpiperazine derivatives designed as homologues of the antipsychotic lead compound LASSBio-579. *Eur J Med Chem* **66**:122–134.

Price DT, Schwinn DA, Lomasney JW, Allen LF, Caron MG, and Lefkowitz RJ (1993) Identification, quantification, and localization of mRNA for three distinct alpha 1 adrenergic receptor subtypes in human prostate. *J Urol* **150**:546–551.

Pupo AS, Uberti MA, and Minneman KP (2003) N-terminal truncation of human alpha1D-adrenoceptors increases expression of binding sites but not protein. *Eur J Pharmacol* **462**:1–8.

Richardson CD, Donatucci CF, Page SO, Wilson KH, and Schwinn DA (1997) Pharmacology of tamsulosin: saturation-binding isotherms and competition analysis using cloned α_1-adrenergic receptor subtypes. *Prostate* **33**:55–59.

Roehrborn CG and Schwinn DA (2004) Alpha1-adrenergic receptors and their inhibitors in lower urinary tract symptoms and benign prostatic hyperplasia. *J Urol* **171**:1029–1035.

Romeiro LA, da Silva Ferreira M, da Silva LL, Castro HC, Miranda AL, Silva CL, Noël F, Nascimento JB, Araújo CV, Tibiriçá E, et al. (2011) Discovery of LASSBio-772, a 1,3-benzodioxole N-phenylpiperazine derivative with potent alpha 1A/D-adrenergic receptor blocking properties. *Eur J Med Chem* **46**:3000–3012.

Sato S, Hatanaka T, Yuyama H, Ukai M, Noguchi Y, Ohtake A, Taguchi K, Sasamata M, and Miyata K (2012) Tamsulosin potently and selectively antagonizes human recombinant $\alpha_{(1A/1D)}$-adrenoceptors: slow dissociation from the $\alpha_{(1A)}$-adrenoceptor may account for selectivity for $\alpha_{(1A)}$-adrenoceptor over $\alpha_{(1B)}$-adrenoceptor subtype. *Biol Pharm Bull* **35**:72–77.

Schwinn DA and Roehrborn CG (2008) Alpha1-adrenoceptor subtypes and lower urinary tract symptoms. *Int J Urol* **15**:193–199.

Tatemichi S, Kobayashi K, Maezawa A, Kobayashi M, Yamazaki Y, and Shibata N (2006) [Alpha1-adrenoceptor subtype selectivity and organ specificity of silodosin (KMD-3213)]. *Yakugaku Zasshi* **126**:209–216.

Walden PD, Gerardi C, and Lepor H (1999) Localization and expression of the alpha1A-1, alpha1B and alpha1D-adrenoceptors in hyperplastic and non-hyperplastic human prostate. *J Urol* **161**:635–640.

Wolters JP and Hellstrom WJG (2006) Current concepts in ejaculatory dysfunction. *Rev Urol* **8** (Suppl 4):S18–S25.

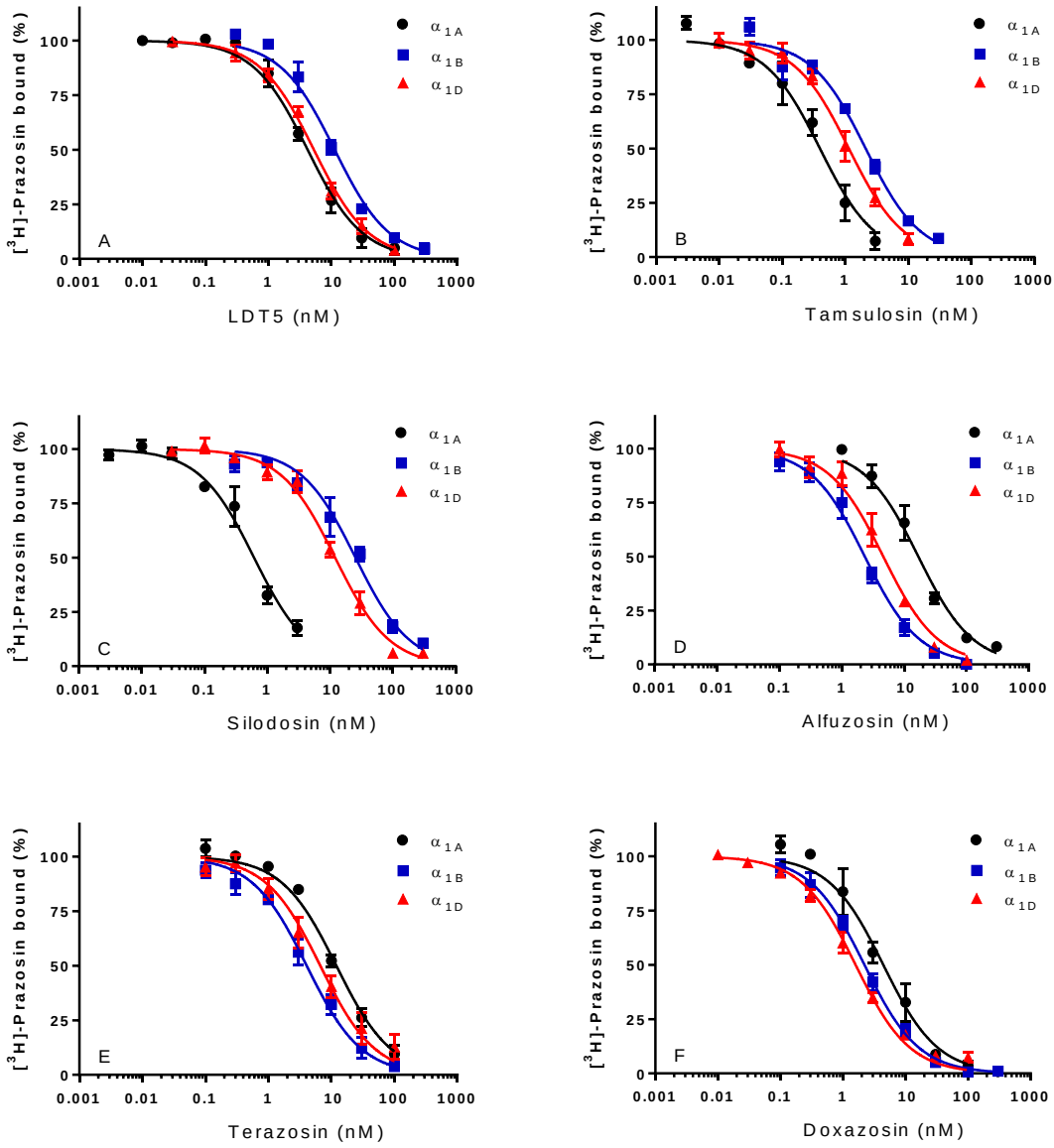
Address correspondence to: François Noël, Laboratory of Biochemical and Molecular Pharmacology, Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Av Carlos Chagas Filho, 373, 21941-902 Rio de Janeiro, Brazil. E-mail: fnoel@pharma.ufrj.br

Supplemental Figure 1

Revisiting the pharmacodynamics uroselectivity of Alpha1-Adrenergic Receptor Antagonists

Bruna Maria Castro Salomão Quaresma, Amanda Reis Pimenta, Anne Caroline Santos da Silva, André Sampaio Pupo, Luiz Antonio S. Romeiro, Claudia Lucia Martins Silva and François Noël*

Journal of Pharmacology and Experimental Therapeutics



Selectivity of the six α_1-AR antagonists for binding to the human α_{1A}- α_{1B}- and α_{1D}-ARs. The membrane preparation of transfected HEK-293 cells (150 µg protein) was incubated for 45 minutes at 30°C in 1 ml medium containing 0.05 nM [^{3}H]-prazosin, Tris–HCl 50 mM (pH 7.4), 1 mM EDTA in the presence or absence of increasing concentrations of the tested compounds. Note that the K_d of [^{3}H]-prazosin were very similar for the three sub-types, being 0.475, 0.354 and 0.577 nM for the α_{1A}-, $\alpha_{1B,}$ and α_{1Dt}-ARs, respectively. The data represent the mean ± SEM of 3-4 independent experiments performed in triplicate.

Supplemental Table 1

Revisiting the pharmacodynamics uroselectivity of Alpha1-Adrenergic Receptor Antagonists

Bruna Maria Castro Salomão Quaresma, Amanda Reis Pimenta, Anne Caroline Santos da Silva, André Sampaio Pupo, Luiz Antonio S. Romeiro, Claudia Lucia Martins Silva and François Noël*

Journal of Pharmacology and Experimental Therapeutics

Table 1: K_i values of the five FDA-approved α_1-AR antagonists for binding to the three human transfected α_1-AR subtypes: overview of literature data.

	***K*$_i$ (nM)**								
α_{1A}	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Tamsulosin	0.012	0.019	0.13	0.04	-	0.63	0.20	-	0.19
Silodosin	0.039	0.036	-	0.20	-	-	-	-	0.44
Alfuzosin	-	-	-	11.5	-	100	10.0	6.31	-
Terazosin	-	-	-	4.90	-	126	-	6.92	8.71
Doxazosin	--	-	-	-	1.99	6.31	3.16	2.75	-
α_{1D}									
Tamsulosin	0.03	0.06	0.18	0.14		0.63	0.16	-	0.05
Silodosin	2.2	2.0	-	5.13		-	-	-	8.71
Alfuzosin	-	-	-	1.99		10.0	3.16	3.98	-
Terazosin	-	-	-	2.57		12.6	-	3.47	1.35
Doxazosin	-	-	-	-	1.66	7.94	3.98	1.66	-
α_{1B}									
Tamsulosin	0.12	0.29	1.92	0.47	-	7.94	1.26	-	0.63

Silodosin	6.5	21	-	5.13	-	-	-	-	10.2
Alfuzosin	-	-	-	1.70	-	12.6	10.0	2.95	-
Terazosin	--	-		2.40	-	12.6	-	1.95	0.46
Doxazosin	-	-	-	-	0.72	6.31	1.00	1.05	-

(1): Tatemichi et al. 2006; (2) Shibata et al., 1995; (3): Kuo et al., 2000; (4) Sato et al., 2012; (5): Hatano et al. 1996; (6): Richardson et al., 1997, (7) Kenny et al., 1996, (8) Forray et al., 1994 (new nomenclature), (9) Ishiguro et al., 2002.