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Pharmacology of Tamsulosin: Saturation-Binding Isotherms and Competition Analysis Using Cloned α_1-Adrenergic Receptor Subtypes

Charlene D. Richardson,[1]* Craig F. Donatucci,[2] Stella O. Page,[1] Katrina H. Wilson,[1] and Debra A. Schwinn[1,2,3]

[1]*Department of Anesthesiology, Duke University, Durham, North Carolina*
[2]*Division of Urology, Department of Surgery, Duke University, Durham, North Carolina*
[3]*Department of Pharmacology, Duke University, Durham, North Carolina*

BACKGROUND. α_1-adrenergic receptors (α_1 ARs) are important in the dynamic component of benign prostatic hyperplasia (BPH). Currently, several α_1AR antagonists are being used in the treatment of BPH.

METHODS. In order to more fully characterize the pharmacology of the α_1AR antagonist tamsulosin, we utilized saturation-binding isotherms with [^{3}H] tamsulosin to determine the Kd of this compound at all three cloned α_1AR subtypes stably expressed in rat-1 fibroblasts. To confirm these results, we performed competition binding experiments, displacing [^{125}I]HEAT with increasing concentrations of alfuzosin, doxazosin, 5-methyl-urapidil, prazosin, tamsulosin, terazosin, and (+)YM617 (stereoisomer of tamsulosin) in the same clonal cell lines.

RESULTS. [^{3}H]tamsulosin binds to cloned α_1AR subtypes with a rank order of affinity of $\alpha_{1a} = \alpha_{1d} > \alpha_{1b}$. Competition experiments confirmed the relative nonselectivity of alfuzosin, doxazosin, and prazosin, but revealed slight $\alpha_{1b} = \alpha_{1d} > \alpha_{1a}$ selectivity for terazosin, and clear $\alpha_{1a} = \alpha_{1d} > \alpha_{1b}$ for (+)YM617 and tamsulosin([−]YM617); $\alpha_{1a} > \alpha_{1d} > \alpha_{1b}$ selectivity for 5-methyl-urapidil was confirmed.

CONCLUSIONS. We conclude that tamsulosin displays selectivity for α_{1a} and α_{1d} ARs. This selectivity may contribute to the tamsulosin efficacy reported in several recent clinical studies in patients with BPH. *Prostate 33:55–59, 1997.* © 1997 Wiley-Liss, Inc.

KEY WORDS: **α-adrenergic antagonists; adrenoceptor; adrenergic receptor; benign prostatic hyperplasia; BPH; prostate**

INTRODUCTION

Benign prostatic hyperplasia (BPH) consists of two components: static (modulated by androgens) and dynamic (smooth muscle contraction mediated by α_1-adrenergic receptors [α_1ARs]) [1–3]. Increasingly it has become apparent that the dynamic component of BPH is responsible for many of the clinical symptoms [4]. cDNAs encoding three α_1AR subtypes (α_{1a}, α_{1b}, and α_{1d}) have been cloned, expressed in cell lines, and characterized pharmacologically [5], and a fourth α_1AR (α_{1L}) has been described pharmacologically [6,7]. α_1AR antagonists are being used in the treatment of BPH [3,8,9]; side effects of α_1AR antagonist therapy include dizziness, orthostatic hypotension, and restlessness. In order to avoid side effects, considerable effort has been spent on the development of prostate-selective α_1AR antagonists. Doxazosin, terazosin, and, in Europe, alfuzosin have been extensively studied and widely used in the treatment of BPH, while tamsulosin is a more recent addition. In order to more fully characterize the pharmacology of the α_1AR antagonist tamsulosin, we utilized saturation-binding isotherms with [^{3}H]tamsulosin to determine the Kd of

*Correspondence to: Charlene Richardson, Ph.D., c/o Debra A. Schwinn, M.D., Department of Anesthesiology, Box 3094, Duke University Medical Center, Durham, NC 27710.

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this compound at all three cloned α_1AR subtypes stably expressed in rat-1 fibroblasts. To confirm these results, we performed competition binding experiments displacing [^{125}I]HEAT with increasing concentrations of alfuzosin, doxazosin, 5-methyl-urapidil, prazosin, tamsulosin, terazosin, and (+)YM617 (stereoisomer of tamsulosin) in the same clonal cell lines.

MATERIALS AND METHODS

Rat-1 Fibroblast Stable Transfections

cDNAs encoding α_{1a} [10], α_{1b} [11], and α_{1d} ARs [12,13] were cloned into pZipNeoSv [14]; transfection into rat-1 fibroblasts was accomplished using calcium phosphate precipitation, as previously described [15]. Transfected rat-1 fibroblasts were grown in monolayers in Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2/95% air at 37°C. Selection was maintained in cells expressing α_1AR subtypes by adding the antibiotic G418 (0.8 mg/ml) to the media. Final clonal cell lines expressed ≈1 pmol α_1AR subtype/mg total protein.

Membrane Preparation

Confluent monolayers of cells stably expressing α_1-adrenergic receptor subtypes were scraped from culture flasks (150 cm^2) into 10 ml TE solution (5 mmol/l Tris-HCl, 5 mmol/l EDTA, pH 7.4). A lysate was prepared with a Brinkman polytron (model PT 3000, setting 8 for 10 sec; Brinkman Instruments, Westbury, NY); after pelleting at 40,000*g* for 15 min (Sorvall SM24 rotor, Sorvall, Wilmington, DE), membranes were resuspended in buffer (150 mmol/l NaCl, 50 mmol/l Tris-HCl, 5 mmol/l EDTA, pH 7.4, containing protease inhibitors [5 μg/ml leupeptin, and 10 μg/ml each of benzamidine and soybean trypsin inhibitor]), quickly frozen, and stored at −70°C. Protein content was determined using a bicinchoninic assay (BCA) with bovine serum albumin (BSA) standards (Pierce, Rockford, IL).

[^{3}H]tamsulosin and [^{125}I]HEAT Binding

Saturation-binding isotherms were generated using increasing concentrations of [^{3}H]tamsulosin (0.2–16 nM) incubated with membranes expressing each α_1AR subtype (5–10 μg protein) in the presence and absence of 1 μM prazosin (to determine nonspecific and total binding, respectively). Saturation curves were performed in the presence of protease inhibitors (at concentrations stated for competition curves) in a total volume of 500 μl at 25°C. Incubations were terminated after 45 min with ice-cold 50 mmol/l Tris-HCl at pH 7.4, and the entire mixture was rapidly filtered over GF/C filters using a Brandel harvester (Biomedical Research & Development Laboratories, Inc., Gaithersburg, MD); filters were dried, and then counted in a scintillation counter. Specific binding was calculated by subtracting nonspecific binding from total binding at each [^{3}H]tamsulosin concentration. Each experiment utilized 6 samples (n = 3 total, 3 nonspecific) per [^{3}H]tamsulosin concentration; 3 independent experiments were performed. [^{125}I]HEAT saturation binding experiments were performed as previously described [16].

Competition Analysis

Competition experiments were performed by incubated membranes expressing each α_1AR subtype (5–10 μg protein) with a Kd concentration (100 pM) of the α_1AR antagonist [^{125}I]HEAT, with increasing concentrations of nonradiolabeled competing ligand (10^{-12}–10^{-3} M) at 25°C in a total volume of 0.25 ml; assay buffer contained 150 mmol/l NaCl, 50 mmol/l Tris-HCl, and 5 mmol/l EDTA, pH 7.4, with protease inhibitors leupeptin (5 μg/ml), benzamidine (10 μg/ml), and soybean trypsin inhibitor (10 μg/ml). Incubations were terminated after 45 min with ice-cold 50 mmol/l Tris-HCl at pH 7.4, and the entire mixture was rapidly filtered over GF/C filters using a Brandel harvester. Filters were dried, and then counted in a gamma counter. Each experiment was performed in triplicate, with 5–8 individual experiments for each ligand investigated.

Materials and Chemicals

[^{125}I]HEAT (specific activity, 2,200 Ci/mmol) was purchased from New England Nuclear (Boston, MA) [^{3}H]tamsulosin (specific activity, 56.3 Ci/mmol) was custom-synthesized by Amersham (Arlington Heights, IL) [17]. The following drugs were gifts of the respective companies: alfuzosin (Synthelabs, Bagneux, France), doxazosin (Pfizer, Sandwich, UK), tamsulosin HCl and (+)YM617 (stereoisomer of tamsulosin) (Yamanouchi Pharmaceutical Co., Tokyo, Japan), and terazosin (Abbott Laboratories, North Chicago, IL). 5-methyl-urapidil was purchased from Research Biochemicals International (Natick, MA) and prazosin from Sigma Chemical Co. (St. Louis, MO).

Data Analysis

Data are presented as mean ± SD, to two significant figures. Both saturation and competition curves were fit using the noniterative regression analysis software, InPlot (Graphpad, San Diego, CA). Nonselectivity was

TABLE I. Kd Values for [^{3}H]tamsulosin and [^{125}I]HEAT at Cloned α_1AR Subtypes as Determined by Saturation-Binding Isotherms*

	K_d (nM)	
	[^{3}H]tamsulosin	[^{125}I]HEAT
α_{1a}AR	0.25 ± 0.12	0.073 ± 0.04
α_{1b}AR	0.69 ± 0.10	0.099 ± 0.06
α_{1d}AR	0.46 ± 0.27	0.085 ± 0.12

*Data are presented as mean ± SD. Each experiment was performed in triplicate, n = 3 individual experiments for [^{3}H]tamsulosin and n = 2 experiments for [^{125}I]HEAT. [^{125}I]HEAT experiments were previously performed in this laboratory [16]. Data are reported to two significant figures.

defined as less than one order of magnitude's difference in affinity for individual α_1AR subtypes.

RESULTS

Characteristics of [^{3}H]tamsulosin and [^{125}I]HEAT Binding to Cloned α_1-Adrenergic Receptor Subtypes Stably Expressed in Rat-1 Fibroblasts

Kd values were derived for [^{3}H]tamsulosin from saturation binding isotherms with membranes from rat-1 cells stably transfected with each α_1AR subtype (Fig. 1, Table I). [^{3}H]tamsulosin recognizes α_1AR subtypes with a rank order of affinity $\alpha_{1a} = \alpha_{1d} \geq \alpha_{1b}$, but the affinity differences are small compared to values obtained from competition binding studies. In contrast, [^{125}I]HEAT recognizes all three α_1AR subtypes with similar affinity (Table I).

Drug Affinities at Cloned α_1AR Subtypes Stably Expressed in Rat-1 Cells

Alfuzosin, doxazosin, 5-methyl-urapidil, prazosin, tamsulosin, (+)YM617, and terazosin competed for [^{125}I]HEAT binding in membranes from rat-1 cells stably expressing cloned α_1-adrenergic receptor subtypes with steep and monophasic curves. While alfuzosin, doxazosin, and prazosin demonstrate similar selectivity for all three α_1AR subtypes, terazosin has slightly higher affinity for α_{1d} and α_{1b} than for α_{1a}ARs (Table II). In contrast, tamsulosin and (+)YM617 are more selective for α_{1d} and α_{1a} than for α_{1b}ARs; additionally, tamsulosin demonstrates higher affinity than that of its (+) isomer ([+]YM617) at each α_1AR subtype (Table II). Finally, 5-methyl-urapidil demonstrates $\alpha_{1a} > \alpha_{1d} > \alpha_{1b}$ subtype selectivity, confirming our previous results for this compound [16].

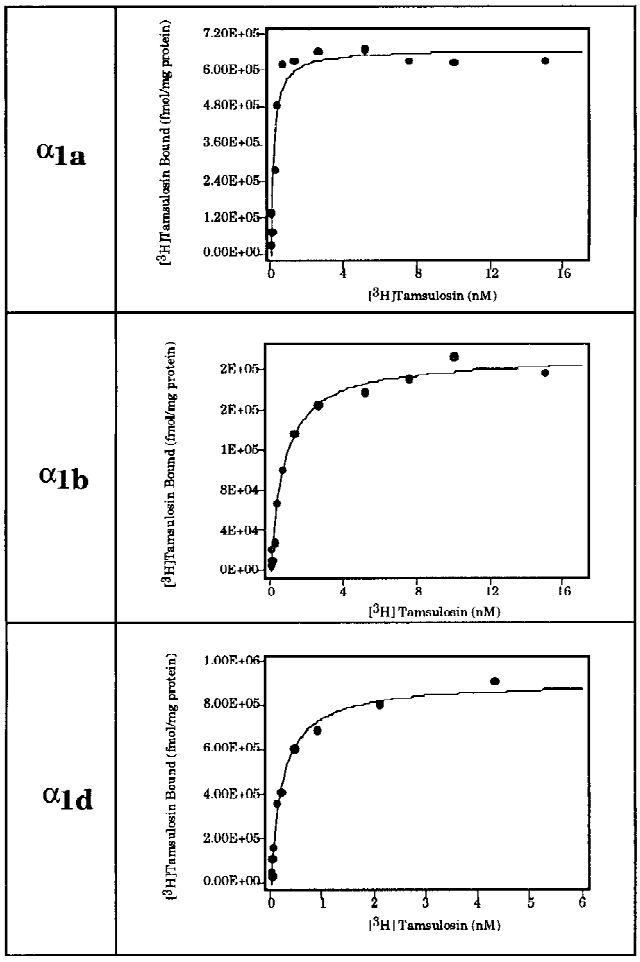

Fig. 1. Examples of saturation curves for [^{3}H]tamsulosin at cloned α_1AR subtypes.

DISCUSSION

Alpha$_1$-adrenergic receptor regulation of prostatic smooth muscle contraction was initially defined by Caine et al. [18] and Raz et al. [19] through isometric tension studies of prostate tissue in the presence of norepinephrine. Additional work by other investigators using various methodologies has supported and confirmed their original findings [1,2,6,19–27]. While the importance of α_1ARs in prostate smooth muscle contraction is clearly defined, the exact α_1AR subtype mediating this response remains an area of intense controversy. To date, cDNAs encoding three α_1AR subtypes (α_{1a}, α_{1b}, and α_{1d}) have been cloned and characterized pharmacologically [10–13], while a putative fourth α_1AR subtype (designated α_{1L}AR due to its low affinity for prazosin) has been described pharmacologically [6,24]. Molecular studies demonstrate α_{1a}AR mRNA to be the predominant subtype in human prostate smooth muscle [28], specifically localized to prostate stroma; this finding has been con-

TABLE II. Affinity for Various α_1AR Antagonists at Cloned α_1AR Subtypes as Determined by Competition Experiments*

	α_{1a}AR ($-pK_i$)	α_{1b}AR ($-pK_i$)	α_{1d}AR ($-pK_i$)
Tamsulosin [(–)YM617]	9.2 ± 0.56	8.1 ± 0.37	9.2 ± 0.31
(+)YM617	7.7 ± 0.42	6.6 ± 0.40	7.3 ± 0.49
Alfuzosin	7.0 ± 0.20	7.9 ± 0.16	8.0 ± 0.54
Doxazosin	8.2 ± 0.68	8.2 ± 0.84	8.1 ± 0.33
5-methylurapidil	8.2 ± 0.14	6.4 ± 0.21	7.2 ± 0.25
Prazosin	8.6 ± 0.19	9.3 ± 0.22	9.5 ± 0.25
Terazosin	6.9 ± 0.12	7.9 ± 0.66	7.9 ± 0.04

*Data are expressed as mean ± SD. Each experiment was performed in triplicate, with n = 5–8 individual experiments for each compound. Data are reported to two significant figures.

firmed at a protein level with ligand binding and contraction studies over the last year by several other laboratories [1,4,29,30]. In spite of these findings, a newly synthesized highly α_{1a}AR-selective drug RS-17053 was recently reported to have low affinity for norepinephrine-induced prostate smooth muscle in contraction experiments, suggesting that another subtype (possibly α_{1L}) might play a role in the prostate as well as in symptomatic BPH [31]. Additionally, ligand binding studies for WB4101, 5-methylurapidil, and HV 723 in prostate tissue also exhibit a low affinity compared to other tissues exhibiting predominately α_{1a}AR subtype [6]. In order to reconcile conflicting molecular evidence identifying α_{1a}AR mRNA in human prostate stroma, but low affinity for RS-17053 in prostate contraction studies, one could postulate that α_{1L}AR is a splice variant of α_1AR. Another possibility is that, although highly selective for α_1AR, RS-17053 might only bind to low-affinity α_{1a}ARs (i.e., the form of the receptor not coupled to the G-protein and therefore not physiologically active); very recent evidence presented by Ford et al. [32] suggests this might be the case. Several more structurally distinct but highly α_{1a}AR-selective compounds need to be tested in the human prostate to resolve this question. Even so, given the current controversy surrounding the possible role (if any) of α_{1L}AR in the human prostate, the selectivity of α_1AR antagonists for individual cloned subtypes remains important.

In this study we report on the selectivity of tamsulosin, its stereoisomer (+)YM617, and other α_1AR antagonists currently used in the therapy of BPH, using a combination of saturation-binding isotherms with [^{3}H]tamsulosin and [^{125}I]HEAT, as well as competition assays with [^{125}I]HEAT in cells stably expressing individual cloned α_1AR subtypes. The advantage of using cloned receptors is the ability to isolate the exact α_1AR subtype/ligand response; this is important, as few tissues (including prostate) express a single α_1AR subtype exclusively. Saturation data demonstrate stereoselectivity between tamsulosin and (+)YM617, with tamsulosin having higher affinity for cloned α_1AR subtypes with the following rank order of affinity: of $\alpha_{1a} = \alpha_{1d} > \alpha_{1b}$. Results from competition experiments demonstrate relative nonselectivity of doxazosin and prazosin, while terazosin exhibits slight selectivity for the α_{1b} over α_{1d} and α_{1a} ARs. While we define alfuzosin as nonselective (i.e., less than one order of magnitude of affinity's difference between subtypes), it is interesting to note that this compound also demonstrates slight selectivity for α_{1b} and α_{1d} ARs. In addition, prazosin, the prototypic nonselective α_1AR antagonist, demonstrates slight α_{1b} and α_{1d} selectivity among nonhuman clones; however, this difference has never reached statistical significance in our laboratory [16]. Selectivity for the reference compound 5-methylurapidil ($\alpha_{1a} > \alpha_{1d} > \alpha_{1b}$) was confirmed. Similar affinity trends have also been observed by other investigators using transient expression in COS cells displacing [^{3}H]prazosin [22,23,33]. Of note, since efficacy at a target organ is complex, depending on both pharmacokinetic (absolute drug concentration in the prostate) as well as pharmacodynamic (receptor subtype-selective) effects, interpretation of clinical efficacy from cloned receptor data must be approached cautiously.

Tamsulosin has recently been shown to completely inhibit phenylephrine-mediated contraction in human prostate hyperplastic tissue at a 10-μM concentration [21]. Combining the results of this study with new knowledge gained using RS-17053 [32], as well as tamsulosin inhibition of in vitro prostate contraction studies [21], clinical effects of tamsulosin appear to result from selectivity for the cloned α_{1a}AR subtype, or possibly the α_{1L}AR. This selectivity may be responsible for the clinical efficacy reported for tamsulosin in several recent clinical trials in patients with BPH [34–36].

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