Tamsulosin Potently and Selectively Antagonizes Human Recombinant $\alpha_{1A/1D}$ -Adrenoceptors: Slow Dissociation from the α_{1A} -Adrenoceptor May Account for Selectivity for α_{1A} -Adrenoceptor over α_{1B} -Adrenoceptor Subtype

Shuichi Sato,^{*,a,†} Toshiki Hatanaka,^a Hironori Yuyama,^b Masashi Ukai,^a Yukiko Noguchi,^a Akiyoshi Ohtake,^a Katsunari Taguchi,^b Masao Sasamata,^a and Keiji Miyata^a

^aApplied Pharmacology Research Laboratories, Drug Discovery Research, Astellas Pharma Inc.; and ^bPharmacology Research Laboratories, Drug Discovery Research, Astellas Pharma Inc.; 21 Miyukigaoka, Tsukuba, Ibaraki 305–8585, Japan Received August 5, 2011; accepted October 10, 2011; published online October 24, 2011

We determined the binding affinity of tamsulosin, a selective α_1 -adrenoceptor antagonist, for human α_1 -adrenoceptor subtypes in comparison with those of other α_1 -adrenoceptor antagonists including silodosin, prazosin, 5-methylurapidil, terazosin, alfuzosin, naftopidil, urapidil and BMY7378. The association and dissociation kinetics of [³H]tamsulosin for recombinant human α_1 -adrenoceptor subtypes were compared with those of [³H]prazosin. Tamsulosin competitively inhibited [³H]prazosin binding to human α_{1A} -, α_{1B} - and α_{1D} -adrenoceptors (pK_i values were 10.38, 9.33, 9.85) indicating 11 and 3.4-fold higher affinities for human α_{1A} -adrenoceptor than those for α_{1B} - and α_{1D} -adrenoceptors, respectively. The affinity of tamsulosin for the human α_{1A} -adrenoceptor was, respectively, 5, 9.9, 38, 120, 280, 400, 1200 and 10000 fold higher than those of silodosin, prazosin, 5-methylurapidil, terazosin, alfuzosin, naftopidil, urapidil and BMY7378, respectively. [³H]Tamsulosin dissociated from the α_{1A} -adrenoceptor slower than from the α_{1B} - and α_{1D} -adrenoceptors ($\alpha_{1B} > \alpha_{1D} > \alpha_{1A}$). Moreover, [³H]tamsulosin dissociated slower than [³H]prazosin from the α_{1A} -adrenoceptor and faster from the α_{1B} - and α_{1D} -adrenoceptors. In conclusion, tamsulosin potently and selectively antagonized $\alpha_{1A/1D}$ -adrenoceptor ligand binding, and slowly dissociated from the α_{1A} -adrenoceptor subtype.

Key words [³H]tamsulosin; α_{1A} -adrenoceptor; dissociation rate; α_1 -adrenoceptor antagonist

Benign prostatic hyperplasia (BPH) is intensified by mechanical obstruction due to prostate hypertrophy and functionally enhanced prostatic smooth muscle contraction.¹⁾ Treatment of the functional component, postulated to result from altered sympathetic tone mediated by α_1 -adrenergic receptors, has involved the use of α_1 -adrenoceptor antagonists.^{2,3)} The adrenoceptors are generally classified into three pharmacological and genetic subtypes designated α_{1A} (α_{1a}), α_{1B} (α_{1b}), and α_{1D} (α_{1d}).⁴⁾ The α_{1A} -adrenoceptor subtype is expressed predominantly in the smooth muscle of the prostate and urethra and is responsible for the smooth muscle contractions that result in voiding symptoms.^{5,6)} The α_1 -adrenoceptor antagonists for treating BPH include prazosin, doxazosin, terazosin, alfuzosin, urapidil, naftopidil and silodosin. Tamsulosin is the first clinically available α_1 -adrenoceptor antagonist showing selectivity for α_{1A} -adrenoceptor for the treatment of BPH with fewer documented cardiovascular side effects than the other available alpha-blocking medications.^{3,7)}

We previously investigated the relationship between the pharmacokinetics of tamsulosin and its inhibitory effect on hypogastric nerve stimulation (HNS)- and phenylephrine-induced prostatic intraurethral pressure (IUP) elevation in anesthetized and conscious male dogs, respectively.^{8,9)} Tamsulosin's effect on HNS-induced IUP response lasted up to 4 h without attenuation and prostatic and urethral concentration remain high, while its plasma concentration decreased to just above the lower limit of quantitation (LLOQ) 4 h after dosing. Similar sustained effects on IUP elevation and prostatic and urethral retention were observed in conscious dogs. One pos-

sible explanation for these observations is that tamsulosin may bind reversibly to the α_{1A} -adrenoceptor, but may dissociate slower from it in the urethra and prostate.

The dissociation and association studies for α_1 -adrenoceptors have been reported using radiolabeled α_1 -adrenoceptor antagonists.^{10–14)} Yamada *et al.*^{10,11)} reported that the dissociation rate constant of [³H]tamsulosin for α_1 -adrenoceptors was less than that of [³H]prazosin in human prostate membrane from BPH patients. These results suggest that tamsulosin may dissociate slower than prazosin from human α_{1A} -adrenoceptors, the most abundant alpha-1 adrenoceptor subtype in human prostate tissue. However, all of these binding kinetic studies were performed using human or animal tissues, and there is no report to our knowledge using recombinant human α_1 -adrenoceptor subtypes. We evaluated the affinities of selective α_1 -adrenoceptor antagonist tamsulosin for recombinant human α_1 -adrenoceptors and compared them with those of other α_1 -adrenoceptor antagonists, prazosin, most classical non-subtype selective α_1 -adrenoceptor antagonist for the treatment of BPH, and silodosin, 5-methylurapidil, terazosin, alfuzosin, naftopidil, urapidil and BMY7378. The binding kinetics (dissociation and association) of [³H]tamsulosin were also evaluated with recombinant human α_1 -adrenoceptors, and in comparison with those of [³H]prazosin.

MATERIALS AND METHODS

Materials Tamsulosin hydrochloride, terazosin hydrochloride and alfuzosin hydrochloride, silodosin, and naftopidil were prepared at Astellas Pharma Inc. (Ibaraki, Japan). Other reagents and their sources are as follows: prazosin hydrochloride, urapidil and phentolamine hydrochloride, Sigma-

[†] Present address: Research Management, Drug Discovery Research, Astellas Pharma Inc.; 21 Miyukigaoka, Tsukuba, Ibaraki 305–8585, Japan.

Aldrich (St. Louis, MO, U.S.A.); BMY7378 dihydrochloride and 5-methylurapidil, Research Biochemicals Inc. (Natick, MA, U.S.A.); [^3H]tamsulosin (1905 GBq/mmol) and [^3H]prazosin (3100 GBq/mmol), PerkinElmer (Boston, MA, U.S.A.). All other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Urapidil and 5-methylurapidil were dissolved in equimolar amounts in hydrochloric acid, silodosin and naftopidil were dissolved in dimethyl sulfoxide (DMSO), and other reagents compounds were dissolved in distilled water. All compounds were diluted with incubation buffer (50 mM Tris-HCl/0.5 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5).

Membrane Preparation COS-1 cells were transiently transfected with the human α_{1A} -, α_{1B} - or α_{1D} -adrenoceptors, and plasma membranes were prepared from each human α_1 -adrenoceptor subtype expressed cell, respectively. Plasma membranes were stored at -80°C until use. We used the Bradford⁽¹⁵⁾ method with a bovine serum albumin (Sigma-Aldrich) standard for determining protein concentrations.

Alpha 1-Adrenoceptor Antagonists—Human α_1 -Adrenoceptor Subtype Binding The α_1 -adrenoceptor binding assays were performed according to the methods described previously with modifications.^{16,17)}

Saturation binding studies for calculating K_d and $B_{\max}$ values were performed in a 500 μL reaction containing [^3H]prazosin concentrations ranging from 0.01 to 2.0 nM, with each plasma membrane suspension reconstituted with incubation buffer, which contained, respectively, 10 μg of α_{1A} or α_{1B} -adrenoceptor or 50–100 μg of α_{1D} -adrenoceptor-enriched membrane proteins prepared from transfected COS-1 cells. Reactions were initiated by adding plasma membrane suspensions, incubated for 45 min at room temperature, and terminated by filtration through Whatman GF/C filters (Packard). After washing filters three times with incubation buffer, the filters were placed in a counting vial containing 5 mL scintillation cocktail (Aquasol[®]-2, Packard) and radioactivity was counted using a TRI-CARB[®], 2200CA (Packard) scintillation counter. Nonspecific binding was determined by including 10 μM phentolamine in the reactions. Competitive binding studies were performed in 500 μL reactions containing [^3H]prazosin (0.2 nM), potential competitors, and incubation buffer. Assay conditions were the same as those described for saturation binding studies. Compounds were tested at concentrations ranging from 10^{-12} to 10^{-5} M, using a common ratio of approximately 3. The IC_{50} 's for [^3H]prazosin were determined by regression analysis of displacement curves. The inhibitory dissociation constant (K_i) was calculated using: $K_i = \text{IC}_{50} / (1 + [L]/K_d)$, where $[L]$ is the radioligand concentration and K_d the dissociation constant of radioligand determined from the Scatchard plot⁽¹⁸⁾ and expressed as the negative logarithm ($\text{p}K_i$).

[^3H]Tamsulosin and [^3H]Prazosin Receptor Binding Kinetics Saturation binding studies were performed to evaluate K_d and $B_{\max}$ values of [^3H]tamsulosin for human α_1 -adrenoceptors as described above. To determine the dissociation rate, 200 pM [^3H]tamsulosin or [^3H]prazosin was incubated with each α_1 -adrenoceptor subtype for 45 min at room temperature at which time 10 μM phentolamine was added and incubation continued for 1 to 180 min. Binding was determined as described above. To determine the association rate, each α_1 -adrenoceptor subtype preparation was incubated in

100 pM [^3H]tamsulosin or 200 pM [^3H]prazosin for 1 to 15 min at room temperature. The reactions were terminated by filtration through Whatman GF/C filters (Packard) and the radioactivity retained on the filter was counted. The association rate (k_{on}), dissociation rate (k_{off}), and $t_{1/2}$ for dissociation of [^3H]tamsulosin and [^3H]prazosin for human α_1 -adrenoceptor binding were calculated as follows:

k_{off} and $t_{1/2}$: $Y = B \cdot \exp(-k_{\text{off}} \cdot X)$, $t_{1/2} = \ln 2 / k_{\text{off}}$ [X : time (min), Y : % binding, specific binding (dpm) at the each sampling point/specific binding (dpm) at time 0, B : % binding at time 0 (initial value=100)].

k_{on} : $Y = B \cdot [1 - \exp(-k_{\text{obs}} \cdot X)]$, $k_{\text{on}} = (k_{\text{obs}} - k_{\text{off}}) / [L]$ [X : time (min), Y : % binding, specific binding (dpm) at the each sampling point/specific binding (dpm) at 15 min, B : % binding at 15 min (initial value: 100), k_{obs} : apparent k_{on}]. Using the k_{on} and k_{off} values calculated as shown above, K_d values of [^3H]tamsulosin and [^3H]prazosin were calculated using the following formula: $K_d = k_{\text{off}} / k_{\text{on}}$

Presentation of Results Values are expressed as the mean $\pm$ standard error of the mean (S.E.M). N represents the number of separate experiments in each group unless otherwise noted. Data were analyzed using SAS software (SAS Institute Inc., NC, U.S.A.). The difference between two groups was analyzed by paired t -test, and the differences between multiple groups were analyzed by Tukey's multiple range test within subject error. Hill slopes were also determined and compared with unity by Student's t -test. A $p < 0.05$ was considered to be statistically significant.

RESULTS

α_1 -Adrenoceptor Antagonist Binding to Membranes Isolated from COS-1 Cells Transiently Expressed with Recombinant Human α_1 -Adrenoceptor Subtypes The binding of [^3H]prazosin to membranes prepared from COS-1 cells transiently transfected with each of the human α_1 -adrenoceptors was saturated in all subtypes. K_d values of [^3H]prazosin for α_{1A} -, α_{1B} - and α_{1D} -adrenoceptors were 0.208 ± 0.037 , 0.063 ± 0.012 and 0.105 ± 0.020 nM, and $B_{\max}$ were 2748 ± 214 , 2385 ± 190 and 134.0 ± 31.2 fmol/mg protein, respectively. Hill slopes for α_{1A} -, α_{1B} - and α_{1D} -adrenoceptor were 0.96 ± 0.05 , 1.00 ± 0.03 and 1.03 ± 0.02 , and statistical differences were not observed when those were compared to unity (Student t -test).

The concentration-inhibition curves of tamsulosin and prazosin are shown in Fig. 1. Table 1 summarizes the K_i values. Tamsulosin and all of the other α_1 -adrenoceptor antagonists tested inhibited the specific binding of [^3H]prazosin to each subtype of human α_1 -adrenoceptors in a concentration-dependent manner (Fig. 1). The $\text{p}K_i$ values of tamsulosin for human α_{1A} -, α_{1B} - and α_{1D} -adrenoceptors were 10.38, 9.33 and 9.85, respectively. Tamsulosin's α_1 -adrenoceptor inhibitory activity was most potent for the α_{1A} -adrenoceptor subtype, and the selectivity of tamsulosin for the α_{1A} -adrenoceptor was 11- and 3.4-fold higher compared with α_{1B} - and α_{1D} -adrenoceptors, respectively. The α_{1A} -adrenoceptor inhibitory activity of tamsulosin was most potent of all the α_1 -adrenoceptor antagonists tested in this study, and 5-, 9.9-, 38-, 120-, 280-, 400-, 1200- and 10000-fold more potent compared with silodosin, prazosin, 5-methylurapidil, terazosin, alfuzosin, naftopidil, urapidil, and BMY7378, respectively (Table 1). The α_1 -adrenoceptor

antagonists, 5-methylurapidil and silodosin, specifically bound the α_{1A} -adrenoceptor, and BMY7378 specifically bound the

α_{1D} -adrenoceptor. In contrast, prazosin, terazosin, alfuzosin, urapidil and naftopidil bound well to each receptor (Table 1).

Fig. 1. Competition of [3 H]Prazosin Binding to Recombinant Human α_{1A} - (●), α_{1B} - (▲) and α_{1D} - (■) Adrenoceptor Subtypes by Tamsulosin (A), and Prazosin (B).

Data are means of 3–6 experiments performed in duplicate.

[3 H]Tamsulosin and [3 H]Prazosin Binding Kinetics for Human α_1 -Adrenoceptor Subtypes The binding of [3 H]-tamsulosin by the human α_1 -adrenoceptor was saturated in all subtypes. K_d values of [3 H]tamsulosin for α_{1A} -, α_{1B} - and α_{1D} -adrenoceptors were 0.077 ± 0.002 , 0.149 ± 0.013 and 0.071 ± 0.008 nM, and B_{max} were 1963 ± 28.0 , 1201 ± 52.7 and 110.6 ± 17.4 fmol/mg protein, respectively. Hill slopes for α_{1A} -, α_{1B} - and α_{1D} -adrenoceptors were 1.02 ± 0.02 , 1.07 ± 0.02 and 0.99 ± 0.02 . These values were not statistically different from 1 (Student *t*-test).

The dissociation kinetics of [3 H]tamsulosin and [3 H]prazosin from human α_1 -adrenoceptors are shown in Fig. 2. The k_{off} and $t_{1/2}$ values are shown in Table 2 and Fig. 3, respectively. [3 H]-Tamsulosin dissociated slower from the α_{1A} -adrenoceptor than from α_{1B} and α_{1D} -adrenoceptors, and the order of the dissociation rates was $\alpha_{1B} > \alpha_{1D} > \alpha_{1A}$ (Fig. 2A, Table 2). In contrast, [3 H]prazosin dissociated faster from the α_{1A} -adrenoceptor than from α_{1D} - and α_{1B} -adrenoceptors (was $\alpha_{1A} > \alpha_{1D} = \alpha_{1B}$) (Fig. 2B, Table 2). These kinetics were confirmed by $t_{1/2}$ analyses (Fig. 3). In the comparison with [3 H]tamsulosin and [3 H]prazosin, [3 H]tamsulosin dissociated slower from the α_{1A} -adrenoceptor and faster from α_{1B} - and α_{1D} -adrenoceptors than [3 H]prazosin (Table 2). The association kinetics of [3 H]tamsulosin and [3 H]prazosin for human α_1 -adrenoceptors are shown in Fig. 4 and k_{on} values are summarized in Table 3. [3 H]Tamsulosin bound faster to α_{1B} -adrenoceptor than to α_{1A} - and α_{1D} -adrenoceptors ($\alpha_{1B} > \alpha_{1D} = \alpha_{1A}$) (Fig. 4A, Table 3). In contrast, no significant differences were observed in the association rates of [3 H]-prazosin for all α_1 -adrenoceptor subtypes (Fig. 4B, Table 3). [3 H]Tamsulosin bound faster to all α_1 -adrenoceptor subtypes compared with [3 H]prazosin (Table 3).

Comparison of K_d 's for [3 H]Tamsulosin and [3 H]Prazosin Receptor Binding The K_d values of [3 H]tamsulosin and [3 H]prazosin determined from saturation and binding kinetics are summarized in Table 4. Except for the K_d for [3 H]tamsu-

Table 1. Drug Binding Affinities for Recombinant Human α_1 -Adrenoceptor Subtypes Expressed in COS-1 Cells

Drug	α_{1A}			α_{1B}			α_{1D}			α_{1B}/α_{1A}	α_{1D}/α_{1A}
	<i>n</i>	pK_i	Hill slope	<i>n</i>	pK_i	Hill slope	<i>n</i>	pK_i	Hill slope		
Tamsulosin	3	10.38 ± 0.04 (1.0)	1.14 ± 0.18	3	9.33 ± 0.02 (1.0)	0.97 ± 0.16	6	9.85 ± 0.08 (1.0)	1.10 ± 0.12	11	3.4
Prazosin	4	9.39 ± 0.10 (1/9.9)	1.44 ± 0.39	4	9.85 ± 0.08 (3.3)	1.12 ± 0.07	6	9.47 ± 0.07 (1/2.4)	1.06 ± 0.18	0.35	0.83
5-Methylurapidil	3	8.80 ± 0.07 (1/38)	0.93 ± 0.04	3	7.53 ± 0.08 (1/63)	1.00 ± 0.06	5	7.91 ± 0.12 (1/88)	1.12 ± 0.20	19	7.9
Terazosin	3	8.31 ± 0.19 (1/120)	1.09 ± 0.27	3	8.62 ± 0.07 (1/5.1)	1.18 ± 0.14	6	8.59 ± 0.07 (1/18)	1.22 ± 0.14	0.49	0.52
Alfuzosin	3	7.94 ± 0.19 (1/280)	0.98 ± 0.04	3	8.77 ± 0.06 (1/3.6)	1.01 ± 0.05	6	8.70 ± 0.15 (1/14)	1.02 ± 0.22	0.15	0.17
Urapidil	3	7.29 ± 0.15 (1/1200)	1.08 ± 0.22	3	6.97 ± 0.05 (1/230)	1.08 ± 0.06	6	6.92 ± 0.11 (1/860)	1.27 ± 0.16	2.1	2.4
BMY7378	3	6.36 ± 0.08 (1/10000)	1.34 ± 0.06	3	7.01 ± 0.14 (1/210)	1.55 ± 0.29	6	8.38 ± 0.10 (1/29)	1.44 ± 0.11	0.23	0.0096
Silodosin	3	9.69 ± 0.15 (1/5.0)	1.41 ± 0.91	3	8.29 ± 0.07 (1/11)	1.13 ± 0.07	3	8.29 ± 0.10 (1/36)	0.99 ± 0.14	25	25
Naftopidil	3	7.78 ± 0.12 (1/400)	1.18 ± 0.01	3	7.78 ± 0.09 (1/35)	1.10 ± 0.04	3	8.14 ± 0.24 (1/51)	1.06 ± 0.19	1.0	0.43

Numbers in parentheses indicate inhibitory potency relative to that of tamsulosin (=1.0). Each value represents the mean $\pm$ S.E.M.

Fig. 2. Receptor-Binding Dissociation Kinetics of $[^3\text{H}]$ Tamsulosin (A) and $[^3\text{H}]$ Prazosin (B)

After $[^3\text{H}]$ labeled ligand binding to membranes had reached equilibrium (for 45 min), dissociation was induced by adding unlabeled phentolamine ($10\mu\text{M}$). The specific binding is expressed as a percentage of binding at equilibrium (at 0 min). Data are the mean $\pm$ S.E.M. of four experiments performed in duplicate in membrane preparations from COS-1 cells transiently transfected with α_{1A} - (●), α_{1B} - (▲) and α_{1D} - (■) adrenoceptor membranes.

Fig. 3. Dissociation Half Lives ($t_{1/2}$) of $[^3\text{H}]$ Tamsulosin (A) and $[^3\text{H}]$ Prazosin (B) for Binding α_{1A} -, α_{1B} - and α_{1D} -Adrenoceptors

The means $\pm$ S.E.M. of four experiments performed in duplicates are displayed. ** $p < 0.01$, statistically significant difference (Tukey's multiple range test).

losin's binding kinetics to the α_{1A} -adrenoceptor subtype, were almost the same, with means $1/2$ to $1/4$ of those determined by saturation binding for all α_1 -adrenoceptor subtypes (Table 4). The K_d value of $[^3\text{H}]$ tamsulosin for the α_{1A} -adrenoceptor subtype was $1/10$ compared to the K_d determined by saturation binding (Table 4).

Fig. 4. Receptor-Binding Association Kinetics of $[^3\text{H}]$ Tamsulosin (A) and $[^3\text{H}]$ Prazosin (B)

Membranes were incubated with $[^3\text{H}]$ tamsulosin (100pM) or $[^3\text{H}]$ prazosin (200pM) for various times. Specific binding is expressed as a percentage of the specific binding at equilibrium (at 15 min). Means $\pm$ S.E.M. of four experiments performed in duplicate are displayed for α_{1A} - (●), α_{1B} - (▲) and α_{1D} - (■) adrenoceptor membranes.

DISCUSSION

In the smooth muscles of the prostate and urethra, the α_{1A} -adrenoceptor subtype is expressed^{5,6)} more abundantly than the α_{1B} and α_{1D} subtypes and is responsible for the dynamic component of obstruction and related voiding symptom. Various α_1 -adrenoceptor antagonists have been developed and used clinically, and tamsulosin is the first clinically available α_1 -adrenoceptor antagonist showing selectivity for α_{1A} -adrenoceptor for the treatment of BPH with fewer documented cardiovascular side effects than the other available alpha-blocking medications.^{3,7)}

We evaluated the affinities of tamsulosin for human α_1 -adrenoceptors compared with those of other α_1 -adrenoceptor antagonists. Tamsulosin exhibited the strongest α_{1A} -adrenoceptor inhibitory activity, and its affinity for the α_{1A} -adrenoceptor was the highest compared with all α_1 -adrenoceptor antagonists tested ($\text{p}K_i = 10.38$). The affinity of tamsulosin among α_1 -adrenoceptor subtypes was highest for the α_{1A} -adrenoceptor, and the selectivity of tamsulosin for the α_{1A} -adrenoceptor was 11- and 3.4-fold higher compared with α_{1B} - and α_{1D} -adrenoceptors, respectively. The drugs 5-methylurapidil and silodosin are selective α_{1A} -adrenoceptor antagonists, BMY7378 is a selective α_{1D} -adrenoceptor antagonist, and prazosin, terazosin, alfuzosin, urapidil, and naftopidil are non-selective α_1 -adrenoceptor antagonists,^{19–23)} consistent with

Table 2. Dissociation Rate Constants (k_{off}) of [^3H]Tamsulosin and [^3H]Prazosin for α_{1A} -, α_{1B} - and α_{1D} -Adrenoceptors

	k_{off} (min^{-1})	
	[^3H]Tamsulosin	[^3H]Prazosin
α_{1A}	$0.023 \pm 0.001^{##}$	0.071 ± 0.004
α_{1B}	$0.351 \pm 0.047^{##}$	0.033 ± 0.002
α_{1D}	$0.052 \pm 0.003^{##}$	0.038 ± 0.003

k_{off} values were calculated from the dissociation study. Values are the mean $\pm$ S.E.M. of four experiments performed in duplicates. $^{##}p < 0.01$, significant difference from [^3H]prazosin group (Student's *t*-test).

Table 3. Association Rate Constant (k_{on}) of [^3H]Tamsulosin and [^3H]Prazosin for α_{1A} -, α_{1B} - and α_{1D} -Adrenoceptors

	k_{on} ($\text{nm}^{-1} \cdot \text{min}^{-1}$)	
	[^3H]Tamsulosin	[^3H]Prazosin
α_{1A}	$3.05 \pm 0.09^{*,##}$	1.36 ± 0.07
α_{1B}	$5.01 \pm 0.37^{##}$	1.29 ± 0.09
α_{1D}	$2.76 \pm 0.13^{*,##}$	1.06 ± 0.10

k_{on} values were calculated from the association study. Values are the mean $\pm$ S.E.M. of four experiments performed in duplicates. $^{**}p < 0.01$, significant difference from k_{on} value on α_{1B} (Tukey's multiple range test). $^{##}p < 0.01$, significant difference from [^3H]prazosin group (Student *t*-test).

Table 4. Comparison of Dissociation Constants (K_d) of [^3H]Tamsulosin and [^3H]Prazosin for Binding α_{1A} -, α_{1B} - and α_{1D} -Adrenoceptors Calculated from Kinetic and Saturation Data

		Kinetics (K)		Scatchard (S)		Ratio (S/K)
		K_d (nM)	(n)	K_d (nM)	(n)	
[^3H]Tamsulosin	α_{1A}	0.007	(4)	0.077 ± 0.002	(4)	10
	α_{1B}	0.070	(4)	0.149 ± 0.013	(4)	2.1
	α_{1D}	0.019	(4)	0.071 ± 0.008	(4)	3.8
[^3H]Prazosin	α_{1A}	0.053	(4)	0.208 ± 0.037	(9)	4.0
	α_{1B}	0.026	(4)	0.063 ± 0.012	(9)	2.4
	α_{1D}	0.036	(4)	0.105 ± 0.020	(10)	2.9

K_d values of kinetic study were calculated from dissociation rate constants (k_{off}) and association rate constants (k_{on}). K_d values of Scatchard analysis were calculated from saturation data. Values are the mean $\pm$ S.E.M. of 4 to 10 experiments performed in duplicate.

our results. We confirmed tamsulosin as a potent and selective $\alpha_{1A/1D}$ -adrenoceptor antagonist in this study.

The binding kinetic studies of radiolabeled α_1 -adrenoceptor antagonist have been reported using α_1 -adrenoceptor membranes prepared from human or animal tissues.^{10–14} To our knowledge, ours is the first study to compare the binding kinetics of [^3H]tamsulosin, an α_1 -adrenoceptor antagonist, to recombinant human α_1 -adrenoceptor subtypes, in comparison with those of [^3H]prazosin. [^3H]Tamsulosin dissociated slower from the α_{1A} -adrenoceptor than from either the α_{1B} - and α_{1D} -adrenoceptors, and [^3H]tamsulosin dissociated slower from the α_{1A} -adrenoceptor and faster from α_{1B} - and α_{1D} -adrenoceptors than [^3H]prazosin. [^3H]Tamsulosin associated with the α_{1B} -adrenoceptor faster than to the α_{1A} - and α_{1D} -adrenoceptors, and [^3H]tamsulosin associated with all α_1 -adrenoceptor subtypes faster than [^3H]prazosin. Data published by Yamada *et al.*^{10,11} suggested that [^3H]tamsulosin would dissociate from human α_{1A} -adrenoceptors slower than [^3H]prazosin. This relationship of the dissociation rates of [^3H]tamsulosin and [^3H]prazosin from α_1 -adrenoceptors was the same as that determined here. The differences in the absolute binding values between ours and Yamada *et al.* may be accounted for by the different receptor sources (for example, transfected *versus* endogenous receptors, α_{1B} - and α_{1D} -adrenoceptors).

In saturation binding experiments, [^3H]tamsulosin had a smaller B_{max} than [^3H]prazosin. Expressed as a percentage of the [^3H]prazosin B_{max} , the [^3H]tamsulosin B_{max} were 71% (α_{1A}), 50% (α_{1B}) and 83% (α_{1D}), respectively. Similar results were reported not only in several rat tissues and human prostate but also in cloned rat (α_{1A} , α_{1B}) and human (α_{1B}) adrenoceptors.^{11,24} The reasons for this difference of B_{max} remain to be determined.

In general, the K_d calculated from kinetics data match the K_d value calculated from saturation binding, if the binding fol-

lows the law of mass action. In order to confirm this, we calculated the ratio of the K_d values. As the result, the K_d value of [^3H]tamsulosin for the α_{1A} -adrenoceptor in kinetics study was lower than the K_d value in saturation study. One possible explanation for this difference is that slow dissociation of [^3H]tamsulosin from the α_{1A} -adrenoceptor ($t_{1/2}$ of ca. 30 min) prevents the attainment of equilibrium in the saturation study (duration of 45 min).²⁵ Equilibrium is closely approached after a time exceeding three-fold the dissociation half life.²⁵

We found that tamsulosin exerted prolonged inhibition of HNS- and phenylephrine-induced IUP elevation, which are generated mainly by α_{1A} -adrenoceptor stimulation in anesthetized and conscious male dogs, respectively.^{8,9} Moreover, prostatic and urethral tamsulosin concentrations were higher than in plasma and correlated well related with effects on IUP, indicating that the sustained effect of tamsulosin on the IUP response appears to be related to the prostatic and urethral retention of tamsulosin. Tamsulosin's slow dissociation from the α_{1A} -adrenoceptor may explain, in part, how tamsulosin is retained in lower urinary tract tissues and its sustained inhibitory effect on prostatic IUP elevation in dogs.^{8,9} A recent study found that the unbound tamsulosin fraction was 0.4% in plasma and 59.1% in prostate indicating, when calculated based on unbound tamsulosin, a ratio of 63 for prostate *versus* plasma concentrations (C_{max}) in BPH patients.²⁶ Tamsulosin is known to show high affinity for α_1 -acid glycoprotein (AGP).²⁷ There is a possibility that AGP concentrations could be variable between plasma and prostate. The differences in local concentration of AGP might be as the driving force for the higher free drug fraction in prostate than in plasma.²⁶ Therefore, highly and freely available unbound tamsulosin in the target tissues (prostate) may also contribute to its long-lasting effect.

We have shown that [^3H]tamsulosin dissociated slower from the α_{1A} -adrenoceptor than from the α_{1B} and α_{1D} -adrenoceptors,

while [^3H]prazosin dissociated faster from the α_{1A} -adrenoceptor than from α_{1D} - and α_{1B} -adrenoceptors. Tamsulosin is widely used to treat BPH and causes fewer hypotensive side effects compared with prazosin and other α_1 -adrenoceptor antagonists.³⁾ We showed the selectivity of tamsulosin for α_{1A} -adrenoceptors was 11-fold higher than for α_{1B} -adrenoceptors. Yamada *et al.*²⁸⁾ reported that tamsulosin has about 12-fold higher affinity for α_1 -adrenoceptors human prostate than human aorta, whereas prazosin and urapidil showed similar affinity for both binding sites. Although the main tamsulosin's decreased hypotensive side effect is considered to be mainly based on its α_{1A} -adrenoceptor-binding selectivity, its slower dissociation rate from α_{1A} -adrenoceptors compared with that for α_{1B} -adrenoceptors may be the other reason. In conclusion, tamsulosin is a potent and selective $\alpha_{1A/ID}$ -adrenoceptor antagonist, and slowly dissociation from α_{1A} -adrenoceptor subtype to a significantly greater extent than from other α_1 -adrenoceptor subtypes.

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