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

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

Key words $[^{3}H]$tamsulosin; $\alpha_{1A}$-adrenoceptor; dissociation rate; $\alpha_{1}$-adrenoceptor antagonist

Benign prostatic hyperplasia (BPH) is intensified by mechanical obstruction due to prostate hypertrophy and functionally enhanced prostatic smooth muscle contraction. Treatment of the functional component, postulated to result from altered sympathetic tone mediated by $\alpha_{1}$-adrenergic receptors, has involved the use of $\alpha_{1}$-adrenoceptor antagonists. The adrenoceptors are generally classified into three pharmacological and genetic subtypes designated $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$. The $\alpha_{1A}$-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. The $\alpha_{1}$-adrenoceptor antagonists for treating BPH include prazosin, doxazosin, terazosin, alfuzosin, urapidil, naftopidil and silodosin. Tamsulosin is the first clinically available $\alpha_{1}$-adrenoceptor antagonist showing selectivity for the $\alpha_{1A}$-adrenoceptor for the treatment of BPH with fewer documented cardiovascular side effects than the other available alpha-blocking medications.

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. 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 possible explanation for these observations is that tamsulosin may bind reversibly to the $\alpha_{1A}$-adrenoceptor, but may dissociate more slowly from it in the urethra and prostate.

The dissociation and association studies for $\alpha_{1}$-adrenoceptors have been reported using radiolabeled $\alpha_{1}$-adrenoceptor antagonists. Yamada et al. reported that the dissociation rate constant of $[^{3}H]$tamsulosin for $\alpha_{1A}$-adrenoceptor was less than that of $[^{3}H]$prazosin in human prostate membrane from BPH patients. These results suggest that tamsulosin may dissociate more slowly than prazosin from human $\alpha_{1A}$-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 $\alpha_{1}$-adrenoceptor subtypes. We evaluated the affinities of selective $\alpha_{1}$-adrenoceptor antagonist tamsulosin for recombinant human $\alpha_{1}$-adrenoceptors and compared them with those of other $\alpha_{1}$-adrenoceptor antagonists, prazosin, most classical non-subtype selective $\alpha_{1}$-adrenoceptor antagonist for the treatment of BPH, and silodosin, 5-methylurapidil, terazosin, alfuzosin, naftopidil, urapidil and BMY7378. The binding kinetics (dissociation and association) of $[^{3}H]$tamsulosin were also evaluated with recombinant human $\alpha_{1}$-adrenoceptors, and in comparison with those of $[^{3}H]$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 phenolamine hydrochloride, Sigma-
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 (50mM Tris–HCl/0.5mM ethylenediaminetetraacetic acid (EDTA), pH 7.5).

**Membrane Preparation** COS-1 cells were transiently transfected with the human α₁A, α₁B or α₁D-adrenoceptors, and plasma membranes were prepared from each human α₁- adrenoceptor subtype expressed cell, respectively. Plasma membranes were stored at −80°C until use. We used the Bradford method with a bovine serum albumin (Sigma-Aldrich) standard for determining protein concentrations.

**α₁-Adrenoceptor Antagonist—Human α₁-Adrenoceptor Subtype Binding** The α₁-adrenoceptor binding assays were performed according to the methods described previously with modifications.

Saturation binding studies for calculating K_d and B_max values were performed in a 500μL reaction containing [³H]prazosin concentrations ranging from 0.01 to 2.0nM, with each plasma membrane suspension reconstituted with incubation buffer, which contained, respectively, 10μg of α₁A or α₁D-adrenoceptor or 50—100μg of α₁D-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 5mL scintillation cocktail (Aquadex®-2, Packard) and radioactivity was counted using a TRI-CARB®, 2200CA (Packard) scintillation counter. Nonspecific binding was determined by including 10μM phentolamine in the reactions. Competitive binding studies were performed in 500μL reactions containing [³H]prazosin (0.2nM), 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 10⁻⁷M, using a common ratio of approximately 3. The IC₅₀’s for [³H]prazosin were determined by regression analysis of displacement curves. The inhibitory dissociation constant (K_i) was calculated using: K_i=IC₅₀/(1+[L]/K_d), where [L] is the radioligand concentration and K_d the dissociation constant of radioligand determined from the Scatchard plot and expressed as the negative logarithm (pK_d).

[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 α₁-adrenoceptors as described above. To determine the dissociation rate, 200pM [³H]tamsulosin or [³H]prazosin was incubated with each α₁-adrenoceptor subtype for 45 min at room temperature at which time 10μM phentolamine was added and incubation continued for 1 to 180min. Binding was determined as described above. To determine the association rate, each α₁-adrenoceptor subtype preparation was incubated in 100pM [³H]tamsulosin or 200pM [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 t₁/₂ for dissociation of [³H] tamsulosin and [³H]prazosin for human α₁-adrenoceptor binding were calculated as follows:

\[ k_{\text{on}} = \frac{X}{t_{1/2}} \quad \text{and} \quad t_{1/2} = \frac{1}{k_{\text{on}}} \]

\[ t_{1/2} = \frac{1}{k_{\text{off}}} \]

Presentation of Results Values are expressed as the mean ± 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

**α₁-Adrenoceptor Antagonist Binding to Membranes Isolated from COS-1 Cells Transiently Expressed with Recombinant Human α₁-Adrenoceptor Subtypes** The binding of [³H]prazosin to membranes prepared from COS-1 cells transiently transfected with each of the human α₁-adrenoceptors was saturated in all subtypes. K_d values of [³H]prazosin for α₁A, α₁B and α₁D-adrenoceptors were 0.208±0.037, 0.063±0.012 and 0.105±0.020nM, and B_max were 2748±214, 2385±190 and 134.0±31.2fmol/mg protein respectively. Hill slopes for α₁A, α₁B and α₁D-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’s 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 α₁-adrenoceptor antagonists tested inhibited the specific binding of [³H]prazosin to each subtype of human α₁-adrenoceptors in a concentration-dependent manner (Fig. 1). The pK_i values for tamsulosin for human α₁A, α₁B and α₁D-adrenoceptors were 10.38, 9.33 and 9.85, respectively. Tamsulosin’s α₁D-adrenoceptor inhibitory activity was most potent for the α₁D-adrenoceptor subtype, and the selectivity of tamsulosin for the α₁A-adrenoceptor was 11- and 3.4-fold higher compared with α₁A- and α₁B-adrenoceptors, respectively. The α₁A-adrenoceptor inhibitory activity of tamsulosin was most potent of all the α₁-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).
Table 1. Drug Binding Affinities for Recombinant Human α₃-Adrenoceptor Subtypes Expressed in COS-1 Cells

<table>
<thead>
<tr>
<th>Drug</th>
<th>α₃</th>
<th>pKᵢ</th>
<th>Hill slope</th>
<th>α₁B</th>
<th>pKᵢ</th>
<th>Hill slope</th>
<th>α₁D</th>
<th>pKᵢ</th>
<th>Hill slope</th>
<th>α₁B/α₃</th>
<th>α₁D/α₃</th>
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</thead>
<tbody>
<tr>
<td>Tamsulosin</td>
<td>3</td>
<td>10.38±0.04</td>
<td>1.14±0.18</td>
<td>3</td>
<td>9.33±0.02</td>
<td>0.97±0.16</td>
<td>6</td>
<td>9.85±0.08</td>
<td>1.10±0.12</td>
<td>11</td>
<td>3.4</td>
</tr>
<tr>
<td>Prazosin</td>
<td>4</td>
<td>9.39±0.10</td>
<td>1.44±0.39</td>
<td>4</td>
<td>9.85±0.08</td>
<td>1.12±0.07</td>
<td>6</td>
<td>9.47±0.07</td>
<td>1.06±0.18</td>
<td>0.35</td>
<td>0.83</td>
</tr>
<tr>
<td>5-Methylurapidil</td>
<td>3</td>
<td>8.80±0.07</td>
<td>0.93±0.04</td>
<td>3</td>
<td>7.53±0.08</td>
<td>1.00±0.06</td>
<td>5</td>
<td>7.91±0.12</td>
<td>1.12±0.20</td>
<td>19</td>
<td>7.9</td>
</tr>
<tr>
<td>Terazosin</td>
<td>3</td>
<td>8.31±0.19</td>
<td>1.09±0.27</td>
<td>3</td>
<td>8.62±0.07</td>
<td>1.18±0.14</td>
<td>6</td>
<td>8.59±0.07</td>
<td>1.22±0.14</td>
<td>0.49</td>
<td>0.52</td>
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<tr>
<td>Alfuzosin</td>
<td>3</td>
<td>7.94±0.19</td>
<td>0.98±0.04</td>
<td>3</td>
<td>8.77±0.06</td>
<td>1.01±0.05</td>
<td>6</td>
<td>8.70±0.15</td>
<td>1.02±0.22</td>
<td>0.15</td>
<td>0.17</td>
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<tr>
<td>Urapidil</td>
<td>3</td>
<td>7.29±0.15</td>
<td>1.08±0.22</td>
<td>3</td>
<td>6.97±0.05</td>
<td>1.08±0.06</td>
<td>6</td>
<td>6.92±0.11</td>
<td>1.27±0.16</td>
<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>BMY7378</td>
<td>3</td>
<td>6.36±0.08</td>
<td>1.34±0.06</td>
<td>3</td>
<td>7.01±0.14</td>
<td>1.55±0.29</td>
<td>6</td>
<td>8.38±0.10</td>
<td>1.44±0.11</td>
<td>0.23</td>
<td>0.0096</td>
</tr>
<tr>
<td>Silodosin</td>
<td>3</td>
<td>9.69±0.15</td>
<td>1.41±0.91</td>
<td>3</td>
<td>8.29±0.07</td>
<td>1.13±0.07</td>
<td>3</td>
<td>8.29±0.10</td>
<td>0.99±0.14</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Naftopidil</td>
<td>3</td>
<td>7.78±0.12</td>
<td>1.18±0.01</td>
<td>3</td>
<td>7.78±0.09</td>
<td>1.10±0.04</td>
<td>3</td>
<td>8.14±0.24</td>
<td>1.06±0.19</td>
<td>1.0</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate inhibitory potency relative to that of tamsulosin (=1.0). Each value represents the mean±S.E.M.
Losin's binding kinetics to the $\alpha_{1A}$-adrenoceptor subtype were almost the same, with means 1/2 to 1/4 of those determined by saturation binding for all $\alpha_1$-adrenoceptor subtypes (Table 4). The $K_d$ value of $[3H]$tamsulosin for the $\alpha_{1A}$-adrenoceptor subtype was 1/10 compared to the $K_d$ determined by saturation binding (Table 4).

DISCUSSION

In the smooth muscles of the prostate and urethra, the $\alpha_{1A}$-adrenoceptor subtype is expressed more abundantly than the $\alpha_{1B}$ and $\alpha_{1D}$ subtypes and is responsible for the dynamic component of obstruction and related voiding symptom. Various $\alpha_1$-adrenoceptor antagonists have been developed and used clinically, and tamsulosin is the first clinically available $\alpha_1$-adrenoceptor antagonist showing selectivity for the $\alpha_{1A}$-adrenoceptor. Tamsulosin exhibited the strongest $\alpha_{1A}$-adrenoceptor inhibitory activity, and its affinity for the $\alpha_{1A}$-adrenoceptor was the highest compared with all $\alpha_1$-adrenoceptor antagonists tested ($pK_i = 10.38$). The affinity of tamsulosin among $\alpha_1$-adrenoceptor subtypes was highest for the $\alpha_{1A}$-adrenoceptor, and the selectivity of tamsulosin for the $\alpha_{1A}$-adrenoceptor was 11- and 3.4-fold higher compared with $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptors, respectively. The drugs 5-methylurapidil and silodosin are selective $\alpha_{1A}$-adrenoceptor antagonists, BMY7378 is a selective $\alpha_{1D}$-adrenoceptor antagonist, and prazosin, terazosin, alfuzosin, urapidil, and naftopidil are non-selective $\alpha_1$-adrenoceptor antagonists, consistent with
our results. We confirmed tamsulosin as a potent and selective α1A/1D-adrenoceptor antagonist in this study.

The binding kinetic studies of radiolabeled α1-adrenoceptor antagonist have been reported using α1-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 α1-adrenoceptor antagonist, to recombinant human α1-adrenoceptor subtypes, in comparison with those of [3H]prazosin. [3H]Tamsulosin dissociated slower from the α1A-adrenoceptor than from either the α1B- and α1D-adrenoceptors, and [3H]tamsulosin dissociated slower from the α1A-adrenoceptor and faster from α1B- and α1D-adrenoceptors than [3H]prazosin. [3H]Tamsulosin associated with the α1A-adrenoceptor faster than to the α1A- and α1D-adrenoceptors, and [3H]tamsulosin associated with all α1-adrenoceptor subtypes faster than [3H]prazosin. Data published by Yamada et al.10,11 suggested that [3H]tamsulosin would dissociate from human α1A-adrenoceptors slower than [3H]prazosin. This relationship of the dissociation rates of [3H]tamsulosin and [3H]prazosin from α1-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, α1B- and α1D-adrenoceptors).

In saturation binding experiments, [3H]tamsulosin had a smaller Bmax than [3H]prazosin. Expressed as a percentage of the [3H]prazosin Bmax, the [3H]tamsulosin Bmax were 71% (α1A), 50% (α1B) and 83% (α1D), respectively. Similar results were reported not only in several rat tissues and human prostate but also in cloned rat (α1A, α1B) and human (α1B) adrenoceptors.11,24 The reasons for this difference of Bmax remain to be determined.

In general, the KD values calculated from saturation binding, if the binding follows the low of mass action. In order to confirm this, we calculated the ratio of the KD values. As the result, the KD value of [3H]tamsulosin for the α1A-adrenoceptor in kinetics study was lower than the KD value in saturation study. One possible explanation for this difference is that slow dissociation of [3H]tamsulosin from the α1A-adrenoceptor (t1/2 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 α1A-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 α1A-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 plasma concentrations (Cmax) in BPH patients.26 Tamsulosin is known to show high affinity for α1-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 α1A-adrenoceptor than from the α1B and α1D-adrenoceptors,

Table 2. Dissociation Rate Constants (kd) of [3H]Tamsulosin and [3H]-Prazosin for α1A-, α1B- and α1D-Adrenoceptors

<table>
<thead>
<tr>
<th></th>
<th>[3H]Tamsulosin</th>
<th>[3H]Prazosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1A</td>
<td>0.023±0.001**</td>
<td>0.071±0.004</td>
</tr>
<tr>
<td>α1B</td>
<td>0.351±0.047**</td>
<td>0.033±0.002</td>
</tr>
<tr>
<td>α1D</td>
<td>0.052±0.003**</td>
<td>0.038±0.003</td>
</tr>
</tbody>
</table>

kd values were calculated from the dissociation study. Values are the mean±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 (ka) of [3H]Tamsulosin and [3H]-Prazosin for α1A-, α1B- and α1D-Adrenoceptors

<table>
<thead>
<tr>
<th></th>
<th>[3H]Tamsulosin</th>
<th>[3H]Prazosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1A</td>
<td>3.05±0.09**</td>
<td>1.36±0.07</td>
</tr>
<tr>
<td>α1B</td>
<td>5.01±0.37**</td>
<td>1.29±0.09</td>
</tr>
<tr>
<td>α1D</td>
<td>2.76±0.13**</td>
<td>1.06±0.10</td>
</tr>
</tbody>
</table>

ka values were calculated from the association study. Values are the mean±S.E.M. of four experiments performed in duplicates. *p<0.01, significant difference from [3H]prazosin group (Student’s t-test).

Table 4. Comparison of Dissociation Constants (KD) of [3H]Tamsulosin and [3H]Prazosin for Binding α1A-, α1B- and α1D-Adrenoceptors Calculated from Kinetic and Saturation Data

<table>
<thead>
<tr>
<th></th>
<th>Kinetics (K)</th>
<th>Scatchard (S)</th>
<th>Ratio (S/K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]Tamsulosin</td>
<td>Kd (nm)</td>
<td>n</td>
<td>Kd (nm)</td>
</tr>
<tr>
<td>α1A</td>
<td>0.007 (4)</td>
<td>0.077±0.002 (4)</td>
<td>10</td>
</tr>
<tr>
<td>α1B</td>
<td>0.070 (4)</td>
<td>0.149±0.013 (4)</td>
<td>2.1</td>
</tr>
<tr>
<td>α1D</td>
<td>0.019 (4)</td>
<td>0.071±0.008 (4)</td>
<td>3.8</td>
</tr>
<tr>
<td>[3H]Prazosin</td>
<td>Kd (nm)</td>
<td>n</td>
<td>Kd (nm)</td>
</tr>
<tr>
<td>α1A</td>
<td>0.053 (4)</td>
<td>0.208±0.037 (9)</td>
<td>4.0</td>
</tr>
<tr>
<td>α1B</td>
<td>0.026 (4)</td>
<td>0.063±0.012 (9)</td>
<td>2.4</td>
</tr>
<tr>
<td>α1D</td>
<td>0.036 (4)</td>
<td>0.105±0.020 (10)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Kd values of kinetic study were calculated from dissociation rate constants (kd) and association rate constants (ka). Kd values of Scatchard analysis were calculated from saturation data. Values are the mean±S.E.M. of 4 to 10 experiments performed in duplicate.
while [3H]prazosin dissociated faster from the α1A- 
adrenoceptor than from α1D- and α1A-adrenoceptors. Tamsulosin is widely used to treat BPH and causes fewer hypotensive side effects compared with prazosin and other α1-adrenoceptor antagonists. We showed the selectivity of tamsulosin for α1A-adrenoceptors was 11-fold higher than for α1D-adrenoceptors. Yamada et al. reported that tamsulosin has about 12-fold higher affinity for α1D-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 α1A-adrenoceptor-binding selectivity, its slower dissociation rate from α1A-adrenoceptors compared with that for α1D-adrenoceptors may be the other reason. In conclusion, tamsulosin is a potent and selective α1A/1D-adrenoceptor antagonist, and slowly dissociation from α1A-adrenoceptor subtype to a significantly greater extent than from other α1- 
adrenoceptor subtypes.

REFERENCES

ethoxyphenoxy]ethyl)[aminol][propyl]2-methoxybenzensulfonamide 
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