Effects of Tamsulosin Metabolites at Alpha-1 Adrenoceptor Subtypes

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ABSTRACT

We have investigated the affinity and selectivity of tamsulosin and its metabolites, M1, M2, M3, M4 and AM1, at the tissue and the cloned alpha-1 adrenoceptor subtypes in the radioligand binding and the functional studies. In the radioligand binding studies, the compounds competed for [3H]prazosin binding to the rat liver and kidney alpha-1 adrenoceptors, with the rank order of potency tamsulosin >> M4 >> M1 >> M2 >> M3 >> AM1 with the latter having a negligible affinity. All compounds differentiated cloned alpha-1 adrenoceptor subtypes with the rank order of potency alpha-1A ≥ alpha-1D > alpha-1B, except for M4 which had the highest affinity for the alpha-1D adrenoceptor. The compounds also concentration-dependently antagonized phenylephrine-induced contractions in the rabbit aorta and prostate. The resulting apparent pA2 values were very similar to those at the cloned rat alpha-1A adrenoceptor. We conclude that most tamsulosin metabolites are high potency antagonists at the alpha-1 adrenoceptors and retain the alpha-1A over the alpha-1B adrenoceptor selectivity of tamsulosin.

Alpha-1 adrenoceptor antagonists are used in the symptomatic treatment of benign prostatic hyperplasia (Oesterling, 1995; Chapple, 1995). It is believed that their beneficial therapeutic effect results from the antagonism of noradrenaline-induced contraction of prostatic smooth muscle that occurs via the alpha-1 adrenoceptors (Hieble et al., 1985). In recent years it has become clear that at least three subtypes of the alpha-1 adrenoceptors exist, which are now designated as alpha-1A (formerly alpha-1c), alpha-1B and alpha-1D (formerly alpha-1a/d) (Hieble et al., 1995; Michel et al., 1995). Among these subtypes the alpha-1A adrenoceptor dominates in the human prostate at the mRNA level (Price et al., 1993; Tseng-Crank et al., 1995), the protein level (Lepor et al., 1993; Michel et al., 1996) and may also be most important for the mediation of contraction (Forray et al., 1994; Marshall et al., 1995). Therefore, it has been suggested that drugs with selectivity for the alpha-1A adrenoceptors may be efficacious in benign prostatic hyperplasia, but may be more tolerable than the nonselective alpha adrenoceptor antagonists (Chapple, 1995).

Tamsulosin is the only alpha-1 adrenoceptor antagonist used clinically in benign prostatic hyperplasia, which is selective for the alpha-1A relative to alpha-1B adrenoceptors, and has an intermediate affinity for the alpha-1D adrenoceptors (Testa et al., 1995; Foglar et al., 1995; Michel et al., 1996). Because in vivo drug effects may also involve the metabolites, we have compared the affinities of tamsulosin and its metabolites M1, M2, M3, M4 and AM1 (fig. 1) at the alpha-1 adrenoceptor subtypes by using competition binding studies with rat tissues (Michel et al., 1993) and the cloned subtypes (Michel and Insel, 1994) as well as functional measurements in the rabbit prostate (Honda et al., 1985a) and the aorta (Honda et al., 1985b).

Materials and Methods

Membrane preparations from the rat liver and the kidney were prepared from male Wistar rats (Lippische Versuchstierzucht, Extertal, Germany) (150–300 g) as described previously in detail (Michel et al., 1993). The expression vector plasmids pCMValpha-1a containing the EcoRI-Pst1 2520 b.p. fragment of the rat alpha-1D adrenoceptor cDNA and pcDV1alpha-1b containing a 2573 b.p. fragment including the entire coding region of the rat alpha-1B adrenoceptor cDNA (Lomasney et al., 1991) were obtained from Dr. R. J. Lefkowitz (Durham, NC). The plasmid pMT2alpha-1c that contains the entire coding region of the rat alpha-1A adrenoceptor (Perez et al., 1994) was obtained from the American Type Culture Collection (ATCC). Three constructs were transfected into COS-1 cells for transient expression by using the diethylaminoethyl-dextran method with the addition of chloroquine and dimethylsulfoxide steps as described previously (Suryanarayana and Kobilka, 1991; Michel and Insel, 1994). Four days after transfection, the cells were harvested, resuspended into ice-cold binding buffer (50 mM Tris and 0.5 mM EDTA, pH 7.5) and homogenized by a TissueMizer for 10 sec at full

ABBREVIATION: Bmax, maximum binding sites.
speed followed twice for 20 sec at ½ speed. The homogenate was centrifuged for 20 min at 50,000 \( \times g \) and the resulting pellet was resuspended in the binding buffer at a concentration of 0.6 to 2 mg/ml.

\[^{3}H\]Prazosin binding to the membrane preparations from the rat tissue or transfected COS-1 cells was performed in the binding buffer (see above) as described previously (Michel et al., 1993). Briefly, 100 \( \mu l \) of membrane suspension were incubated in a total volume of 1 ml with the indicated \[^{3}H\]prazosin concentrations for 45 min at 25°C. The incubations were terminated by rapid vacuum filtration over Whatman GF/C filters. Nonspecific binding was defined as binding in the presence of 10 \( \mu M \) phenolamine. In competition experiments, a \[^{3}H\]prazosin concentration of \( \approx 200 \mu M \) was used.

For the functional experiments, male albino rabbits (weight, 2.3–4.8 kg) were obtained from Kitayama Labes Co. (Nagano, Japan). Experiments were performed as described previously (Honda et al., 1985a,b) at 37°C in 30-ml organ baths containing Krebs-Henseleit solution of the following composition (millimolar): NaCl, 118.4; KCl, 4.7; KH$_2$PO$_4$, 1.2; MgSO$_4$, 1.2; CaCl$_2$, 2.5; NaHCO$_3$, 25.0; and glucose, 11.1. For experiments on the rabbit aorta, helical strips of 2 \( \times \) 30 mm were used. For the experiments on the prostate, tissue strips (3 mm wide and 15 mm long) were prepared in the transverse direction. Aortic and prostatic specimens were equilibrated under a resting tension of 2 and 1 g, respectively, for 1 to 2 hr; these resting tensions were chosen because they allow maximum tension development (Honda et al., 1985a,b). Phenylephrine (3 \( \mu M \)) was administered repeatedly until responsiveness became stable. After vigorous washout, cumulative phenylephrine concentration-response curves were generated with half-logarithmic concentration increments. After the washout, the tissues were equilibrated with the antagonists for 30 min and another concentration-response curve was constructed. For each antagonist, except for AM-1, three to four (rabbit aorta) or two to four (rabbit prostate) concentrations were tested. Apparent \( pA_2 \) values were determined at each antagonist concentration from the shift of the concentration-response curve by the Furchgott equation

\[
pA_2 = -\log[A] + \log(\text{EC}_{50}/\text{EC}_{50}) - 1
\]

where \( \text{EC}_{50} \) and \( \text{EC}_{50}' \) are the half-maximally effective agonist concentrations in the absence and presence of the antagonist, respectively, and \([A]\) is the concentration of the antagonist. \( pA_2 \) values from all antagonist concentrations were then averaged. Schild analysis was not performed routinely in the present study, but we have shown previously that tamsulosin as well as prazosin, yohimbine and phenolamine yield Schild-regressions with slopes not significantly different from unity when large numbers of preparations are tested under these conditions (Honda et al., 1985a,b, 1987).

\[^{3}H\]Prazosin (specific activity, 70–80 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Tamsulosin and its metabolites were synthesized by Yamanouchi. Phenylephrine was a gift of Ciba Geigy (Basel, Switzerland). Phenylephrine HCl was obtained from Tokyo Kasei (Tokyo, Japan).

Data are the means ± S.E.M. of the number (n) of experiments. Statistical significance of drug affinity differences at the \( \alpha \)-adrenoceptor subtypes was determined in two ways: first, competition binding experiments were analyzed by fitting mono- and biphase sigmoidal curves to the experimental data; a biphase fit was accepted only if it resulted in a significant improvement of the fit as judged by an F test. Second, drug affinities at the cloned \( \alpha \)-adrenoceptor subtypes were compared by a one-way analysis of variance; if this indicated that the variance between groups was significantly greater than that within groups, individual groups were compared by the Tukey-Kramer multiple comparison tests. In all tests, a P value < .05 was considered significant. Statistical analysis was performed by the InStat program (GraphPAD Software, San Diego, CA). All curve fitting procedures were performed by using the lnPlot program (GraphPAD Software).

**Results**

\[^{3}H\]Prazosin was bound to the rat liver membranes with a \( K_d \) of 132 ± 36 \( \mu M \) and a \( B_{\text{max}} \) of 123 ± 13 fmol/mg of protein (n = 3). Except for the AM1, all test compounds competed for \[^{3}H\]prazosin binding to the rat liver membranes with steep and monophasic competition curves (fig. 2; table 1). The order of potency in the rat liver was tamsulosin \( \sim M4 > M1 > M2 \sim M3 \gg AM1 \). Thus, AM1 in concentrations up to 100 \( \mu M \) competed for less than 50% of the \[^{3}H\]prazosin binding. Similarly high concentrations of AM1 also competed for only a small fraction of \[^{3}H\]prazosin binding in the rat kidney or with any of the cloned \( \alpha \)-adrenoceptor subtypes. Thus, the AM1 appears to have very low affinity for all subtypes of

![Fig. 1. Chemical structure of tamsulosin and its metabolites. Throughout the study, M4 was used in its racemic form whereas pure optical (minus)-isomers were used for all other compounds.](image)

![Fig. 2. Competition of tamsulosin and its metabolites for the \[^{3}H\]prazosin binding to the rat liver membranes. Data are the means of three experiments. Error bars are not shown for clarity of presentation, but usually were 5 to 10%. A numerical analysis of these data is shown in table 1.](image)
the rat \( \alpha_1 \) adrenoceptors and will not be discussed any further.

\( ^{[3}H \)Prazosin was bound to the rat kidney membranes with a \( K_d \) of 110 \( \pm \) 15 \( \mu \)M and a \( B_{\text{max}} \) of 31 \( \pm \) 3 fmol/mg of protein \( (n = 3) \). In the rat renal membranes, all test compounds competed for the \( ^{[3}H \)prazosin binding with shallow competition curves that were explained much better by a two-rather than a one-site model (fig. 3; table 1). The order of potency in the renal membranes at both the high and the low affinity sites was similar to that in the rat liver, and all compounds recognized a similar percentage of the high affinity sites, \( i.e. \) approximately 35 to 50%.

\( ^{[3}H \)Prazosin was bound to the cloned rat \( \alpha_1 \)-A, \( \alpha_1 \)-B and \( \alpha_1 \)-D adrenoceptors with \( K_d \) values of 263 \( \pm \) 36, 176 \( \pm \) 22 and 27 fmol/mg of protein \( (n = 3) \), respectively. All test compounds competed for the \( ^{[3}H \)prazosin binding to the cloned rat \( \alpha_1 \) adrenoceptor subtypes (table 2). The order of potency at each subtype was similar to that observed in the rat liver or the kidney. All compounds were subtype-selective, having their lowest affinity at the \( \alpha_1 \)-B adrenoceptor. Most compounds recognized the cloned \( \alpha_1 \) adrenoceptor subtypes with the order of potency \( \alpha_1 \)-A \( \approx \) \( \alpha_1 \)-D \( > \) \( \alpha_1 \)-B. The metabolite M4, however, had the highest affinity at the \( \alpha_1 \)-D adrenoceptor.

In the rabbit aorta and the prostate phenylephrine elicited contractions with potencies (EC\(_{50}\)) of approximately 0.3 and 4 \( \mu \)M, respectively. The metabolite AM1 did not affect the phenylephrine-induced contraction in either tissue in concentrations up to 1 \( \mu \)M. In contrast, tamsulosin and its other metabolites caused concentration-dependent parallel shifts of the phenylephrine concentration-response curve to the right toward higher concentrations without affecting its maximal response. From these right shift, drug affinities (apparent \( pA_2 \) values) could be calculated, which are depicted in table 3.

**Discussion**

Presently, tamsulosin is the only \( \alpha_1 \) adrenoceptor antagonist in clinical use that discriminates \( \alpha_1 \) adrenoceptor subtypes (Testa et al., 1995; Foglar et al., 1995; Michel et al., 1996). Inasmuch as drug effects *in vivo* may result in part from their metabolites, it is important to know whether tamsulosin metabolites also are subtype-selective \( \alpha_1 \) adrenoceptor antagonists. Studies with \( ^{14}C \)tamsulosin have suggested that unchanged tamsulosin accounts for 91% of the recovered radioactivity from plasma at \( C_{\text{max}} \) and for 74%
of the area under the curve, indicating that the metabolites do not play a major role for the in vivo effects of tamsulosin; the compounds M1, M2, M3, M4 and AM1 have been identified as major tamsulosin metabolites (Soeishi et al., 1996). To evaluate further a possible role of metabolites in the in vivo effects of tamsulosin, the present study has determined the potency of tamsulosin metabolites at α1-adrenoceptors and their selectivity for α1-adrenoceptor subtypes in the radioligand binding and functional assay system in comparison with the parent compound, tamsulosin. In the radioligand binding experiments, we have used the rat liver as a tissue containing a homogeneous population of the α1B adrenoceptors (Han and Minneman, 1991; Büscher et al., 1996) and the rat kidney as a tissue containing a mixed population of multiple α1-adrenoceptor subtypes (Michel et al., 1993); additionally, the cloned rat α1A, α1B and the α1D adrenoceptors (Schwinn et al., 1990; Lomasney et al., 1991; Perez et al., 1994) were studied upon transient expression in COS cells. Whereas we have reported previously an excellent correlation between drug affinities at the rat liver and the cloned α1B adrenoceptors for a large number of compounds (Büscher et al., 1996), in the present study drug affinities at the cloned α1B adrenoceptor were generally somewhat lower than in the liver. Moreover, in the present study tamsulosin affinities at all cloned subtypes were somewhat lower than in our previous studies (Michel and Insel, 1994). Although we have no good explanation for these discrepancies, it should be noted that reported affinity estimates at the cloned subtypes underly a surprisingly large variation that considerably exceeds that in the native tissues (Michel et al., 1995). For the functional tests, phenylephrine-induced contractions were studied in a model of α1A adrenoceptors, rabbit prostate (Testa et al., 1993, 1995). Additionally, we have used the rabbit aorta in which phenylephrine-induced contraction mainly occurs via an α1A adrenoceptor in our hands (Honda et al., 1985b, 1987), but which has been demonstrated by other investigators to contain the multiple α1-adrenoceptor subtypes (Vargas and Gorman, 1995).

The biochemically or functionally determined affinities of tamsulosin in the various models in the present study are consistent with values obtained in our laboratories in previous studies (Honda et al., 1985a,b; Michel et al., 1993; Büscher et al., 1996). Overall, the affinities observed at the cloned α1A adrenoceptor subtypes are well within the range of values obtained in other laboratories (Perez et al., 1994; Horie et al., 1994; Foglar et al., 1995; Testa et al., 1995).

Thus, in a balanced view of published data, tamsulosin appears to be 10- to 20-fold-selective for the α1A relative to the α1B adrenoceptors with intermediate affinities at the α1D adrenoceptors.

### Table 3

Affinity of tamsulosin and its analogs in the rabbit aorta and prostate

<table>
<thead>
<tr>
<th></th>
<th>Aorta</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamsulosin</td>
<td>9.71 ± 0.09</td>
<td>9.87 ± 0.13</td>
</tr>
<tr>
<td>AM1</td>
<td>&lt;6</td>
<td>&lt;6</td>
</tr>
<tr>
<td>M1</td>
<td>8.75 ± 0.04</td>
<td>8.96 ± 0.10</td>
</tr>
<tr>
<td>M2</td>
<td>8.44 ± 0.02</td>
<td>8.70 ± 0.12</td>
</tr>
<tr>
<td>M3</td>
<td>8.54 ± 0.03</td>
<td>8.83 ± 0.03</td>
</tr>
<tr>
<td>M4</td>
<td>9.59 ± 0.06</td>
<td>9.64 ± 0.07</td>
</tr>
</tbody>
</table>

The tamsulosin metabolites generally showed the rank order of potency tamsulosin ≈ M4 > M1 > M2 ≈ M3 ≫ AM1 in the radioligand binding and functional assays. Thus, the metabolite M4 has an affinity at the α1-adrenoceptors similar to that of tamsulosin itself and therefore might contribute to the sympatholytic effect of tamsulosin; this contribution, however, is unlikely to be large due to the low abundance of the metabolite. The metabolites M1, M2 and M3 have somewhat lower affinity for the α1-adrenoceptors than tamsulosin; therefore, it may be expected that these metabolites contribute even less to the pharmacological in vivo profile in humans. The metabolite AM1 has only a negligible α1-adrenoceptor affinity and is highly unlikely to contribute to the pharmacological tamsulosin effects in vivo.

Due to the high α1-adrenoceptor affinity of some tamsulosin analogs, it is interesting to know whether these metabolites retain the subtype-selectivity profile of tamsulosin. Our data in the rat kidney demonstrate that indeed all tested tamsulosin analogs (except for the very low affinity AM1) are sufficiently subtype-selective to yield biphasic competition curves and to allow discrimination of the α1-adrenoceptors in this tissue. However, it should be noted that the rat kidney most likely contains more than two α1-adrenoceptor subtypes (Michel et al., 1993), and thus the high- and low-affinity sites in the rat kidney may not exactly reflect the α1A and α1B adrenoceptor affinities.

Our studies on the cloned α1A-adrenoceptor subtypes confirm that all tamsulosin analogs (except AM1) have significantly higher affinity for the α1A relative to the α1B adrenoceptors, and that the degree of selectivity for all of them is similar to that of tamsulosin itself. High-potency functional antagonism of the metabolites was also confirmed in two functional models, the rabbit aorta and prostate, which at least in our hands mainly involve the α1A adrenoceptors (Honda et al., 1985b, 1987); however, the rabbit aorta may also involve other subtypes according to published data (Vargas and Gorman, 1995). Most tamsulosin analogs, similar to tamsulosin itself, have intermediate affinity for the α1D adrenoceptors. A notable exception is M4, that has a higher affinity for the α1D than for the α1A adrenoceptors. M4 also differs from the other compounds of this study, because it is the only compound in which the benzene sulfonylamine rather than the phenoxyming has been modified. In particular, in the M4 the methoxy group of the benzenesulfonylamine ring has been replaced by a hydroxy group, yielding a more catecholamine-like structure. Thus, the M4 has certain similarities with the endogenous catecholamines adrenaline and noradrenaline that also are somewhat selective for the α1D relative to the α1A and α1B adrenoceptors among the rat or the human α1-adrenoceptor subtypes (Forray et al., 1994; Laz et al., 1994; Michel and Insel, 1994; Schwinn et al., 1995). From these data, it can be hypothesized that the α1A/α1B adrenoceptor selectivity of tamsulosin is encoded in the phenoxy ring moiety of the molecule and that this selectivity is not affected by the additional hydroxylation or substitution of the ethoxy by a methoxy group. In contrast,
hydroxylation of the benzenesulfonamide moiety selectively increases the alpha-1D adrenoceptor affinity of the molecule. Whether tamsulosin and its metabolites functionally behave as an alpha-1D adrenoceptor antagonist has not been tested directly to our knowledge. However, tamsulosin is a high-potency antagonist for the contraction of the rat aorta (Eltzé, 1994; van der Graaf et al., 1996), a bona fide model of alpha-1D adrenoceptors (Vargas and Gorman, 1995).

In conclusion, we have demonstrated that most tamsulosin metabolites are high-affinity antagonists at the alpha-1 adrenoceptors and retain the subtype-selectivity profile of their parent compound, tamsulosin.

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References


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Tamsulosin Metabolites


