α1-Adrenoceptor Subtype in the Rat Prostate Is Preferentially the α1A Type

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ABSTRACT—α1-Adrenoceptors in the rat prostate were characterized by a binding assay using the newly synthesized radioligand [3H]-YM617 (5-[2-[[2-ethoxyring(n)-3H](o-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide HCl) and an in vitro assay. Specific [3H]-YM617 binding in the rat prostate was saturable and of high affinity (KD = 61.5 ± 5.9 pM) with 23.2 ± 6.9 fmol/mg of protein as the maximal number of binding sites (Bmax). α-Adrenoceptor agonists and antagonists inhibited the binding of the radioligand with the following order of effectiveness: YM617 > prazosin = bunazosin > WB4101 > 5-methylurapidil = phenoxybenzamine > phentolamine > S(+) -isomer of YM617 > yohimbine > norepinephrine > phenylephrine > methoxamine. α1-Adrenoceptors in the rat prostate preferred the R(-)-isomer of YM617 to the S(+) -isomer. Preincubation with chlorethylclonidine (CEC; 10⁻⁵ M, 10 min) just slightly changed the Bmax value for [3H]-YM617 without changing the KD value in the prostate; however, CEC reduced the Bmax in the aorta. In the isolated tissue, pretreatment with CEC (10⁻⁵ M, 10 and 30 min) time-dependently shifted to the right the dose-response curve for phenylephrine and decreased the maximal contraction of aortas induced by phenylephrine, but did not shift or decrease those of prostates. The present results indicate that the α1-Adrenoceptors in the rat prostate are mainly CEC-insensitive (α1A), whereas those in the aorta are CEC-sensitive (α1B).

Keywords: Prostate (rat), α1-Adrenoceptor subtype

α-Adrenoceptor subtypes in the lower urinary tract have been investigated (1-5). The prostate is closely related to the urethra both anatomically and embryologically (6). The prostate smooth muscle of both human and animals have been reported to contain α-adrenoceptors which mediate contractions (7, 8), and their α-adrenoceptor subtype is the α1 type (3, 9).

Benign prostatic hypertrophy (BPH) is a progressive enlargement of the prostate, occurring in men with increasing frequency with advancing age. The symptoms such as bladder outlet obstruction observed in these patients result from constriction of the urethra by the enlarging prostate which surrounds it (10). The urinary obstruction in benign prostatic hypertrophy is decreased effectively by an administration of α1-adrenoceptor antagonists such as phenoxybenzamine (11, 12), prazosin (13, 14), YM-12617 (15) and YM617 (16). Kawabe et al. (16) have reported that phenoxybenzamine, prazosin and YM617 improved some of the symptoms such as bladder outlet obstruction in BPH; however, although phenoxybenzamine and prazosin lowered the blood pressure, YM617 failed to lower blood pressure. They have suggested that the α1-adrenoceptors in the arteries are different from those in the prostate.

A binding study with radiolabeled prazosin has been utilized to characterize α1-adrenoceptors in central and peripheral tissues by a number of investigators since the development of the method by Hornung et al. (17) and Karliner et al. (18). Recently, [3H]-YM617 was synthesized and it was characterized to be an α1-selective radioligand by Sudoh et al. (19) and Yazawa et al. (20).

Recent studies have revealed that α1-adrenoceptors are actually a group of heterogeneous proteins that can be subtype based on their affinity for various antagonists (21) or their primary structure (22, 23). According to the classification of Minneman et al. (21), two subtypes of α1-adrenoceptor can be identified based on the receptor affinity for the competitive antagonist WB4101 and the irreversible antagonist chloroethylclonidine. Johnson and Minneman (24) reported that chloroethylclonidine (CEC), a reactive derivative of clonidine, selectively inactivated a subset of the α1-adrenoceptor that was insensitive to WB4101, whereas the other subset was sensitive to WB4101. These findings suggest that α1-adrenoceptors may exist as a heterogeneous population of proteins that can be differentiated by their affinity for specific antagonists.
binding sites labeled with $[^{25}I]$-BE2254 in rat cerebral cortex, suggesting the presence of two types of binding sites that differed in their sensitivity to CEC. It is tempting to speculate that CEC-sensitive ($\alpha_{1b}$) and -insensitive ($\alpha_{1a}$) binding sites represent the two subtypes of $\alpha_1$-adrenoceptors.

The aim of the present study was to elucidate the $\alpha_1$-adrenoceptor subtypes ($\alpha_{1a}$ and $\alpha_{1b}$) of the rat prostate in comparison to those in the aorta using $[^3H]$-YM617, CEC and some $\alpha$-adrenoceptor agonists and antagonists.

MATERIALS AND METHODS

Tissue preparation

Male Sprague-Dawley rats (250–400 g, SLC, Shizuoka) were anesthetized with diethylether, and the prostate and aorta of each rat were rapidly removed. Tissues were finely minced with scissors and homogenized in 10 vol. of ice-cold homogenization buffer (0.25 M sucrose containing 50 mM Tris-HCl, pH 7.5) with a Polytron (Kinematica, Lucerne, Switzerland). The homogenate was centrifuged at 600 $\times$ g for 15 min at 4°C. The supernatant was filtered through a single layer of nylon mesh and recentrifuged at 40,000 $\times$ g for 20 min at 4°C. The resulting pellets were washed twice with ice-cold incubation buffer (50 mM Tris-HCl, pH 7.5, containing 10 mM MgCl$_2$). The final pellet was resuspended with 3–10 vol. of ice-cold incubation buffer and stored at −80°C until use.

CEC pretreatment

Aliquots of the resuspended membrane preparations were incubated with or without 10 $\mu$M CEC for 10 min at 37°C in Tris-HCl buffer. Reactions were stopped by the addition of ice-cold incubation buffer followed by centrifugation at 40,000 $\times$ g for 20 min at 4°C. The resulting pellets were washed twice with ice-cold incubation buffer (50 mM Tris-HCl, pH 7.5, containing 10 mM MgCl$_2$). The final pellet was resuspended with 3–10 vol. of ice-cold incubation buffer and stored at −80°C until use.

Binding assay

The binding assay was carried out as follows: $\alpha_1$-adrenoceptor density was determined in saturation experiments by incubating membrane aliquots (approximately 20–300 $\mu$g protein) with increasing concentrations of $[^3H]$-YM617 (0.02–3 nM) in a final volume of 0.5 ml for 20 min at 25°C. In the competition experiments, a single concentration (0.4 nM) of $[^3H]$-YM617 and 5 to 9 concentrations of antagonists were used. Incubation was terminated by rapid filtration through Whatman GF/C filters (Maidstone, Kent, UK) using a Brandel cell harvester (Gaithersburg, MD, USA). The filter was then rinsed 3 times with 3-ml aliquots of ice-cold incubation buffer. Radioactivity retained on the filters was counted by a liquid scintillation counter (2000CA, Packard, Meriden, CT, USA). Nonspecific binding was determined in the presence of 10 $\mu$M phentolamine. The protein content of each membrane suspension was measured by the method of Bradford.

Isolated tissues

Ventral prostates and thoracic aortas of male Sprague-Dawley rats (300–400 g) were quickly excised and placed in oxygenated Krebs solution. While maintained at room temperature in this buffer, adherent fatty and connective tissues were removed. Aortas were cut into equal-sized ring segments (5 mm in length). In the aorta preparation, the endothelium of each segment was removed by rubbing. Isometric contraction was recorded under a loading tension of 0.5 g for the prostate and 1 g for the aorta, respectively. Both tissues were suspended in 10-ml organ baths containing Krebs solution warmed to 37°C and aerated with a mixture of 95% O$_2$ and 5% CO$_2$. The composition of the Krebs solution was: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO$_4$, 2.5 mM CaCl$_2$, 11.1 mM dextrose, 25.0 mM NaHCO$_3$ and 1.2 mM KH$_2$PO$_4$ dissolved in distilled and demineralized water. The tissues were attached to isometric force-displacement transducers (SB-1T, Nihon Kohden Co., Tokyo) connected to ink oscillographs (MC 6621, Graphtec, Tokyo) through carrier amplifiers (AP-621G, Nihon Kohden). Equilibration was undertaken for 1 or 2 hr before the addition of drugs. Submaximal contractions were first elicited by repeated concentrations of $1 \times 10^{-6}$ M phenylephrine until constant responses were obtained. Cumulative concentration-response curves for phenylephrine were then constructed by increasing the bath concentration of the agonist approximately threefold. The tissues were exposed to CEC for 10 or 30 min. After the respective time, tissues were extensively washed by fresh Krebs solution (usually 12 times). Because the cumulative concentration-response curves under these conditions could not be re-constructed in the same preparation of the prostate, the control and CEC-treated experiments were done in different preparations of both tissues.

Data analyses

Results are expressed as the mean ± S.E.M. or the mean with 95% confidence limit. Comparisons between values from different groups were evaluated by analysis of variance. Probabilities of <5% (P < 0.05) were considered significant. Regression lines were calculated by the least squares method.

Analysis of binding data was performed as previously described (26). IC$_{50}$ values, the concentration required to inhibit specific binding by 50%, were computed by logit-log analysis from the following equation (27):

$$\log([B0-Bi]/[Bi-Bn]) = n[\log(\text{antagonist concentration} - \log(\text{IC}_{50}))]$$
where Bo and Bi are the binding in the absence and presence of the antagonist to be tested, respectively; Bn is the nonspecific binding, and n is a slope factor identical to the Hill coefficient. When test drugs compete with radioligands at the receptor site, the data can be analyzed on the basis of competition theory as follows: If the Hill coefficient is unity, the binding site of the drug being tested is composed of a single population, thereby making possible the computation of the inhibition constant (Ki) according to the following equation:

$$Ki = \frac{IC_{50}}{1 + \frac{[3H\text{-ligand}]}{KD}}$$

where KD is the dissociation constant of the radioligand.

**Materials**

[3H]-YM617 (2.08 TBq) was specially synthesized by Amersham (Tokyo) for Yamanouchi Pharmaceutical Co., Ltd. (Tokyo). YM617, R(-)-5-[2-[[2(o-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide HCl, and its optical enantiomer; 5-methylurapidil; and bunazosin were prepared by Yamanouchi Pharmaceutical Co., Ltd. Phenylephrine HCl and norepinephrine HCl (Tokyo Kasei, Tokyo); prazosin HCl, phentolamine HCl, yohimbine HCl, methoxamine HCl and Tris (hydroxymethyl) aminomethane (trizma base, Sigma Chemical Co., St. Louis, MO, USA); and WB4101, 2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane HCl and CEC 2HC1 (Research Biochemicals, Inc., Natic, MA, USA) were purchased from their respective sources.

**RESULTS**

[3H]-YM617 binding in the rat prostate was concentration-dependent and highly specific. Figure 1 illustrates the specific and nonspecific binding of [3H]-YM617 at the concentration of 0.02 to 3.0 nM in the rat membrane. Nonspecific binding of [3H]-YM617 to the membrane increased linearly with increasing concentration of the ligand. However, the specific binding was saturable, and Scatchard analysis revealed that this binding was monocomponent with a high affinity (K0 = 61.5 pM) and a low capacity (Bmax = 23.2 f mol/mg protein) (Table 1).

Pharmacological binding properties of α-adrenoceptor agonists and antagonists were determined from competition binding curves for [3H]-YM617 in the rat prostate membrane. Three α-adrenoceptor agonists and 9 α-adrenoceptor antagonists were tested for their inhibitory activity against [3H]-YM617 binding in the prostate. Based on the inhibitory curves, the rank order of displacement of [3H]-YM617 binding was as follows: YM617 > prazosin = bunazosin > WB4101 > 5-methylurapidil = phenoxybenzamine > phentolamine > S(+) -isomer of YM617 > yohimbine > norepinephrine > phentolamine > methoxamine in the rat prostate (Fig. 2), and their pKi values are listed in Table 2. YM617 was the most potent inhibitor of the compounds tested against [3H]-YM617 binding in this membrane. Prazosin showed almost the same pKi values in both types of membranes (10.01 in the prostate, n = 6; 10.15 in the aorta, n = 4), but YM617 was approximately 10 times more potent in the prostate (pKi = 10.95, n = 4) than in the aorta (pKi = 9.90, n = 3) (Fig. 3). Pretreatment of membrane preparations with 10 μM

**Table 1.** Effect of CEC pretreatment (10⁻⁷ M for 10 min) on [3H]-YM617 binding sites in the rat aorta and prostate

<table>
<thead>
<tr>
<th></th>
<th>Bmax (fmol/mg protein)</th>
<th>K0 (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>CEC</td>
</tr>
<tr>
<td>Aorta</td>
<td>36.6±6.6</td>
<td>22.9±5.4</td>
</tr>
<tr>
<td>Prostate</td>
<td>23.2±6.9</td>
<td>20.6±5.8</td>
</tr>
</tbody>
</table>

Data are the mean±S.E.M. of 5–8 experiments.
CEC for 10 min caused slightly different effects on specific \(^{[3]H}\)-YM617 binding sites between the prostate and aorta. This treatment decreased the apparent B\(_{\text{max}}\) for ligands by 40% in the aorta, but almost no change in the prostate (Fig. 4). There was little change in the K\(_D\) value for \(^{[3]H}\)-YM617 in the CEC-treated tissues (Table 1 and Fig. 4).

### Table 2. Inhibition of \(^{[3]H}\)-YM617 binding sites by \(\alpha\)-adrenoceptor antagonists and agonists in the rat prostate

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>[^{[3]H}]-YM617</th>
<th>pK(_I)</th>
<th>Hill coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>YM617</td>
<td>4</td>
<td>10.95 (10.93 - 10.97)</td>
<td>1.02 (0.88 - 1.16)</td>
</tr>
<tr>
<td>(S^+)-isomer</td>
<td>4</td>
<td>8.28 (8.27 - 8.30)</td>
<td>0.88 (0.79 - 0.97)</td>
</tr>
<tr>
<td>Prazosin</td>
<td>6</td>
<td>10.01 (10.00 - 10.02)</td>
<td>0.84 (0.76 - 0.91)</td>
</tr>
<tr>
<td>Bunazosin</td>
<td>3</td>
<td>10.06 (10.02 - 10.11)</td>
<td>0.90 (0.75 - 1.06)</td>
</tr>
<tr>
<td>WB4101</td>
<td>4</td>
<td>9.74 (9.72 - 9.76)</td>
<td>0.81 (0.67 - 0.96)</td>
</tr>
<tr>
<td>5-Methylurapidil</td>
<td>4</td>
<td>8.94 (8.93 - 8.95)</td>
<td>0.84 (0.74 - 0.90)</td>
</tr>
<tr>
<td>Phenoxybenzamine</td>
<td>4</td>
<td>8.93 (8.91 - 8.95)</td>
<td>0.79 (0.73 - 0.86)</td>
</tr>
<tr>
<td>Phenolamine</td>
<td>3</td>
<td>8.70 (8.68 - 8.73)</td>
<td>1.05 (0.88 - 1.22)</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>3</td>
<td>6.43 (6.34 - 6.52)</td>
<td>0.79 (0.61 - 0.97)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>4</td>
<td>6.04 (6.03 - 6.05)</td>
<td>0.68 (0.62 - 0.75)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>4</td>
<td>5.73 (5.71 - 5.74)</td>
<td>0.68 (0.61 - 0.74)</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>4</td>
<td>5.43 (5.42 - 5.44)</td>
<td>0.73 (0.66 - 0.79)</td>
</tr>
</tbody>
</table>

Data are the mean and the 95% confidence limit of the indicated number of experiments (n).

Fig. 2. Inhibition of specific \(^{[3]H}\)-YM617 binding to the rat prostate by YM617, prazosin, bunazosin, phenoxybenzamine, WB4101, 5-methylurapidil, phenolamine, the \(S^+\)-form of YM617, yohimbine, norepinephrine, phenylephrine and methoxamine. Competitive binding experiments of \(^{[3]H}\)-YM617 (0.4 nM) binding were performed in the absence and presence of five to nine concentrations of various \(\alpha\)-adrenoceptor agonists and antagonists. Each point represents the mean ± S.E.M. of three to six experiments.

Fig. 3. Inhibition of specific \(^{[3]H}\)-YM617 binding to the rat prostate and aorta by YM617 and prazosin. Competitive binding experiments of \(^{[3]H}\)-YM617 (0.4 nM) binding were performed in the presence of YM617 or prazosin in rat aorta and prostate. Each point represents the mean ± S.E.M. of three to six experiments.
Fig. 4. Chlorethylclonidine (CEC) inactivation of [3H]-YM617 binding sites. Crude membranes from the rat aorta (A) and prostate (B) were incubated with 10 μM CEC or without CEC for 10 min. Reactions were stopped by dilution with cold buffer and centrifugation. After resuspension, saturation isotherms of specific [3H]-YM617 binding were determined in the control (○) and CEC-pretreated (■) tissues. Each point is the mean of triplicate determinations from three to five experiments.

Fig. 5. The effect of chlorethylclonidine (CEC) on phenylephrine induced contractions of the rat aorta (A) and prostate (B). Thoracic aorta and prostate were prepared and treated as described in Methods. The tissue was pretreated without (Control) or with 10 μM chlorethylclonidine (CEC) for 10 (left) or 30 (right) min, washed intensively and concentration-response curves for phenylephrine determined. Each point is the mean ± S.E.M. of four to six observations.
Phenylephrine concentration-dependently produced tension in the smooth muscle of the rat aorta and prostate. Pretreatment of the aorta with 10 μM CEC for 10 and 30 min shifted the dose-response curve to the right and reduced the maximal response with a pretreatment-time-dependency; however, the pretreatment with CEC did not affect the binding in the prostate membranes (Fig. 5). This result showed that the α₁-adrenoceptors in the aorta were CEC-sensitive, but those in the prostate were not.

DISCUSSION

The major finding of the present study is that the α₁-adrenoceptor subtype of the rat prostate is mainly the α₁A type. We elucidated the subtype by two approaches: one is that we used selective α₁A adrenoceptor agonists and antagonists; for example, methoxamine (28), WB4101 (21), 5-methylurapidil (29) and YM617 (20, 30). The other approach was the use of CEC as an inactivator of α₁B-adrenoceptors (21).

Comparing the values for the dissociation constant (Kᵰ, Kᵩ) of agonists and antagonists has been employed as one of the most reliable techniques for differentiating receptors and receptor subtypes (31). From the results of [³²P]-BE2254 binding to various tissues of rats, pKᵰ values for WB4101 are 9.33–9.88 at the α₁A-adrenoceptor and 8.08–8.40 at the α₁B-adrenoceptor (21). The pKᵩ value of WB4101 in the prostate was 9.74 in the present study. This value is similar to the value for α₁A-adrenoceptors but different from the value for α₁B-adrenoceptors. [³H]-5-Methylurapidil bound to α₁A-adrenoceptors in the rat brain membrane with a pKᵩ value of 9.05 but did not bind to purified liver cell membrane (α₁B), and 5-methylurapidil inhibited [³H]-5-methylurapidil binding to the α₁A-adrenoceptors with a pKᵩ value of 9.13 (29). The pKᵩ value for 5-methylurapidil in the prostate was 8.94 in the present study. This value is near that for α₁A-adrenoceptors.

YM617 was an approximately 10 times more potent inhibitor of the adrenoceptors in the prostate than those in the aorta, because its pKᵩ value in the prostate was 10.95 and that in the aorta was 9.90. However, prazosin was not selective for adrenoceptors in the aorta and the prostate. Kawabe et al. (16) observed that prazosin and YM617 improved symptoms such as bladder outlet obstruction in BPH; however, prazosin lowered blood pressure, but YM617 failed to lower it. The selectivity for adrenoceptors in the prostate seems to provide one of explanations for this observation. As mentioned below, there appeared to be species difference. Because of this difference, it would be very important to obtain results from human tissues.

According to most recent congress abstracts, the α₁-adrenoceptor subtypes of the prostate of rabbits (19), dogs (33) and humans have been reported (34–36). Sudoh et al. (19) reported that CEC reduced Bmax values for the [³H]-YM617, [³H]-prazosin and [³H]-WB4101 binding sites in the aorta, heart and liver of rabbits, but not in the prostate of rabbits, suggesting that the population of α₁-adrenoceptors in rabbit aorta and prostate is preferentially the α₁A type. Chapple et al. (34) reported that WB4101 (10⁻⁸–3 × 10⁻⁷ M) produced a dose-dependent shift to the right of the dose-response curves for norepinephrine in human prostate; the application of CEC (10⁻⁵–10⁻⁴ M) not only resulted in a similar shift to the right but also resulted in a reduction of maximal contractile responses of up to 75%. Through binding studies, Tang et al. (35) found that the dominant α₁A-receptor in human prostate is the α₁-subtype. Furthermore, Suzuki et al. (36) reported that specific [³H]-prazosin binding in crude membranes of human prostate and aorta was saturable and of high affinity (Kᵩ=0.2–0.4 nM). CEC treatment partially inhibited (20–30%) specific [³H]-prazosin binding in both tissues, thereby suggesting the coexistence of α₁-adrenoceptor subtypes. Prazosin and YM617 competed with [³H]-prazosin for the binding sites
in human prostate and aorta. The inhibitory effect (IC_{50}) of prazosin was identical in these tissues (0.68 and 0.52 nM, respectively), but that for YM617 was approximately 14 times more potent in the prostate (0.11 nM) than in the aorta (1.39 nM). This selectivity agrees with our result in the rat prostate and aorta.

In this study, YM617 showed 10-fold selectivity for the prostate compared with the aorta, but similar K_D values of [1^H]-YM617 was obtained in the prostate and aorta. This discrepancy might be explained by other possible classifications of a_1-adrenoceptor subtype. Tsuchihashi et al. (37) reported the existence of two a_1-adrenoceptor subtypes in rat brain (a_1_{high} and a_1_{low}) in experiments using phenoxybenzamine. Furthermore, another subclassification of a_1-adrenoceptor has been proposed, in which three distinct binding sites were termed a_1_{a1B}, a_1_{a1L} and a_1_{aIN} according to their antagonist affinity and susceptibility to CEC (38). On the other hand, four subtypes (a_1_{a1A}, a_1_{a1B}, a_1_{a1C} and a_1_{aIN}) have been cloned (22, 23, 39, 40). However, the relationship between a_1_{a1H} and a_1_{low}-subtypes and a_1_{aIN} and a_1_{a1B}-subtypes has not yet been clarified. So this question remains to be answered.

In summary, the a_1-adrenoceptors in the rat prostate are mainly CEC-insensitive (a_1_{a1A}), whereas those in the aorta are CEC-sensitive (a_1_{a1B}).

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