Comparison of Displacemental Potencies of Terazosin Enantiomers for $\alpha_1$-Adrenoceptor Subtypes

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The present study was designed to assess displacemental potencies of terazosin and its isomers for $\alpha_{1_{\text{High}}}$ and $\alpha_{1_{\text{Low}}}$ adrenoceptor subtypes in rat brain, heart, bovine prostate and canine aorta using a radioligand binding assay method. Although no significant difference in pK$_I$ values of each terazosin isomer for $\alpha_{1_{\text{High}}}$ in canine aorta and rat brain were observed, the displacemental potency of (S-)terazosin for those in rat heart and bovine prostatic was stronger than that of (R+)-isomer ($p < 0.01$). On the other hand, only in $\alpha_{1_{\text{Low}}}$ subtypes of bovine prostate was stronger displacemental potency of (S-)terazosin than (R+) isomer ($p < 0.05$) observed. Thus, these results imply that there is a different affinity between (S-)terazosin and (R+) isomer on the $\alpha_{1_{\text{High}}}$ in bovine prostate and rat heart and $\alpha_{1_{\text{Low}}}$ in bovine prostatic.

Keywords $\alpha_1$-adrenoceptor subtype; terazosin enantiomer; displacemental potency; binding assay method

Terazosin, which is known as a drug for the treatment of hypertension and benign prostatic hyperplasia, contains a chiral center in the tetrahydrofuran residue. This chiral center gives the optical activity in terazosin, which is allowed to exist as (S-) and (R+) isomers.1,3

Many tissues are known to contain $\alpha_1$-adrenoceptors. Subtypes of this receptor have been classified as $\alpha_{1_A}$, $\alpha_{1_B}$,2-8 $\alpha_{1_C}$,9 $\alpha_{1_D}$,10 and $\alpha_{1_L}$, $\alpha_{1_L}$, $\alpha_{1_N}$,11 Our previous reports also described the existence of two kinds of $\alpha_1$-adrenoceptor subtypes in rat brain,12 heart,13 bovine prostate14 and canine aorta,15 using the radioligand binding assay method. One subtype, $\alpha_{1_{\text{High}}}$, showed higher binding affinity for [3H]prazosin than the other subtype, $\alpha_{1_{\text{Low}}}$, in each tissue type.

Thus, this study was designed to compare the displacemental potencies of (rac)terazosin and its isomers for each $\alpha_1$-adrenoceptor subtype ($\alpha_{1_{\text{High}}}$ and $\alpha_{1_{\text{Low}}}$) in rat brain, heart, bovine prostate and canine aorta by radioligand binding assay.

MATERIALS AND METHODS

Materials [3H]Prazosin (76.2 Ci/mmol) was purchased from New England Nuclear Corporation, Ltd. and was stored at -20°C. Prazosin was purchased from Funakoshi. Bunazosin (Eisai), (rac)terazosin and the enantiomers (Abbott Laboratories) were generously donated by each company. All compounds used in the present study were diluted appropriately with deionized distilled water and stored at 4°C.

Preparation of a Crude Membrane-Enriched Fraction

Crude membrane-enriched fraction from rat brain,12 rat heart,13 bovine prostate14 and canine aorta15 were each prepared by the method described previously. All tissues were removed, were frozen in liquid nitrogen and stored at -80°C until used. In brief, the brain, heart and prostate were defrosted at room temperature and minced in either 10 mM Tris-HCl buffer containing 250 mM sucrose, pH 7.6 (brain and heart) or 50 mM Tris-HCl buffer containing 10 mM MgCl$_2$, pH 7.4 (prostate). The aorta was crushed into a fine powder in liquid nitrogen and was suspend-
ed in 10 mM Tris-HCl buffer containing 250 mM sucrose, pH 7.6.15 These suspensions were homogenized using a Polytron homogenizer and filtered through 1 (aorta) or 4 (brain, heart and prostate) layers of gauze. The filtrations of brain and heart were centrifuged for 30 min at 40000 g, while those of prostate and aorta were centrifuged for 5 min at 100 g and 10 min at 1000 g, respectively. The supernatants were then centrifuged for 30 min at 40000 g. All resulting pellets were rinsed once with the incubation buffer (120 mM Tris-HCl, pH 7.4) and homogenized in the incubation buffer using a glass homogenizer. The membrane fractions were then immediately frozen in liquid nitrogen and stored at -80°C until used. Protein concentration was determined by the method of Lowry et al.16 using bovine serum albumin as a standard.

Binding Assay

The displacemental potencies of terazosin enantiomers for $\alpha_1$-adrenoceptor subtypes in all tissues were examined following the method described in previous papers.12-15 In brief, [3H]prazosin was used as a radioligand for the $\alpha_1$-adrenoceptor in every tissue. [3H]Prazosin concentrations used for the assessment of drugs by displacemental experiments for $\alpha_{1_{\text{High}}}$ and $\alpha_{1_{\text{Low}}}$ were as follows: 0.04, 0.5 nM (rat brain), 0.1, 0.6 nM (rat heart), 0.1, 0.5 nM (bovine prostate) and 0.01, 0.3 nM (canine aorta). The assessments of drugs for $\alpha_{1_{\text{Low}}}$, however, were carried out in the presence of phenoxybenzamine (0.1 nM for rat brain and 1.0 µM for heart) or bunazosin (1.0 nM for bovine prostate and canine aorta). As previously reported,12-15 the Scatchard plot determined under these conditions coincided with the line of $\alpha_{1_{\text{Low}}}$ and suggested that these drugs in this concentration could completely displace [3H]prazosin binding on the $\alpha_{1_{\text{High}}}$ subtype. Protein concentrations in the incubation medium were as follows: 0.1 mg (rat brain), 0.15 mg (rat heart and canine aorta) and 0.2 mg (bovine prostate). After incubation at 23°C for 30 min, the medium was rapidly filtered under vacuum through glass fiber filters (Whatman GF/C) using Automatic Cell Harvester Labomash (LM-101, Labo Science). Resulting filters were added to 1 ml of toluene-triton based scintillation fluid and left for more than 8 h. The radioactivity
remaining in the filters was counted by a scintillation counter (Packard 2200 Tri-Carb Scintillation Analyzer). The specific binding of $[^3H]$prazosin was defined as the difference between the total binding and the nonspecific binding in the presence of 10 $\mu$M phenolamine. The values of inhibition constants ($K_i$) were calculated by the method reported$^{17}$ using the $Kd$ value of each tissue.$^{12-15}$

RESULTS AND DISCUSSION

Figure 1 and Table I show typical curves and $pK_i$ values of (rac)terazosin and its isomers for $\alpha_{1\text{High}}$ in each tissue. In all tissues, except for canine aorta and rat brain, the curve of $(R+)$terazosin was shifted more to the right than the curves of the other isomers. The $(S-)$terazosin had a stronger displacement potency for $\alpha_{1\text{High}}$ than the $(R+)$isomer ($p<0.01$). On the other hand, as the curves of these compounds observed in canine aorta and rat brain were consistently almost overlapping, $pK_i$ values of terazosin isomers for $\alpha_{1\text{High}}$ in these two tissues coincided with each other. The displacental potencies of (rac)terazosin were intermediate between $(S-)$ and $(R+)$terazosin. The fact that only $\alpha_{1\text{High}}$ in canine aorta and rat brain could not recognize an optical difference between $(S-)$ and $(R+)$terazosin suggests that the binding characteristics of $[^3H]$prazosin to $\alpha_{1\text{High}}$ subtypes and/or the molecular structure of $\alpha_{1\text{High}}$ in rat heart and bovine prostate may be different from those in canine aorta and rat brain.

Figure 2 and Table II show typical displacental curves and $pK_i$ values of (rac)terazosin and its isomers for $\alpha_{1\text{Low}}$. The curve for $\alpha_{1\text{Low}}$ in bovine prostate gave the same result as that for $\alpha_{1\text{High}}$ in which only the curve of $(R+)$terazosin shifted to the right. Also observed in bovine prostate $\alpha_{1\text{Low}}$ was a stronger displacental potency of $(S-)$terazosin than of $(R+)$isomer. However, in rat brain, heart and canine aorta, no significant difference between $(S-)$ and $(R+)$isomers was detected. In addition, the recognition site of $\alpha_{1\text{Low}}$ for terazosin

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

**Fig. 1. Displacement Curves of (rac)Terazosin (□), (S-)Isomer (●) and (R+)Isomer (○) for $\alpha_{1\text{High}}$-Adrenoceptor Subtypes in Bovine Prostate (A), Canine Aorta (B), Rat Brain (C) and Rat Heart (D)**

**Table I. $pK_i$ Values of Terazosin and Its Isomers for $\alpha_{1\text{High}}$-Adrenoceptor Subtypes in Various Tissues**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Bovine prostate</th>
<th>Canine aorta</th>
<th>Rat brain</th>
<th>Rat heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin</td>
<td>10.64 ± 0.16 (3)</td>
<td>10.38 ± 0.23 (4)</td>
<td>10.60 ± 0.13 (3)</td>
<td>11.14 ± 0.04 (4)</td>
</tr>
<tr>
<td>Bunazosin</td>
<td>9.87 ± 0.24 (4)</td>
<td>9.73 ± 0.24 (4)</td>
<td>9.79 ± 0.24 (3)</td>
<td>9.95 ± 0.03 (3)</td>
</tr>
<tr>
<td>(S-) Terazosin</td>
<td>8.29 ± 0.13 (6)</td>
<td>9.37 ± 0.11 (7)</td>
<td>9.06 ± 0.11 (6)</td>
<td>9.18 ± 0.11 (5)</td>
</tr>
<tr>
<td>(rac) Terazosin</td>
<td>8.25 ± 0.12 (6)</td>
<td>9.22 ± 0.24 (11)</td>
<td>8.92 ± 0.22 (6)</td>
<td>9.15 ± 0.10 (8)</td>
</tr>
<tr>
<td>(R+) Terazosin</td>
<td>7.16 ± 0.23 (9)</td>
<td>8.95 ± 0.24 (7)</td>
<td>8.58 ± 0.26 (9)</td>
<td>8.42 ± 0.19 (8)</td>
</tr>
</tbody>
</table>

Values in parentheses indicate the number of experiments. Data are the mean ± S.E.
enantiomers in bovine prostate may be different from that in other tissues.

In the past few years, $\alpha_1$-adrenoceptors have been subclassified into $\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1C}$ and $\alpha_{1D}$ \cite{10} or $\alpha_{1H}$, $\alpha_{1L}$ and $\alpha_{1M}$ \cite{11} by radioligand binding assay and pharmacological method. Although the distinction between these subclassifications is not yet clear, it is becoming accepted that $\alpha_1$-adrenoceptors in many tissues can be classified into at least the 4 subtypes. Furthermore, the elucidation of pharmacological profiles of drugs at the receptor subtype level may lead to development of new drugs for the treatment of disease. As terazosin enantiomers could be distinguished by displacemtnal potencies in several tissues, the method presented here may be of importance in clarifying the pharmacological effects of terazosin.

Coates et al.\cite{8} suggested that one subtype, $(\alpha_{1A})$ of the $\alpha_1$-adrenoceptor was involved in the contraction of the prostate. In addition, Satoh et al.\cite{19} indicated that norepinephrine-induced contraction is mediated through both $\alpha_{1A}$ and $\alpha_{1B}$-subtypes. We also reported that $pK_1$ values of $\alpha_1$-adrenoceptor blocking agents appear to correlate with the $pK_1$ values of $\alpha_{1H}$ binding sites obtained from radioligand binding assay.\cite{14}

Thus, these $\alpha_1$-adrenoceptor subtypes participate in smooth muscle contraction and terazosin enantiomers showed high $pK_1$ values on them.

Lomasny et al.\cite{8} reported the isolation of cDNAs encoded for $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor from rat cerebral cortex and for $\alpha_{1C}$ from bovine brain. Elucidation of amino acid sequence of these $\alpha_1$-adrenoceptors, however, is currently limited to only a few tissues. The subtypes of $\alpha_1$-adrenoceptor in tissues used in the present study are $\alpha_{1H}$ (high affinity to prazosin) and $\alpha_{1L}$ (low affinity to prazosin). We were unable to determine whether or not the structure and/or pharmacological characteristic of the $\alpha_{1H}$ (or $\alpha_{1L}$) subtype in the four tissues used here are the same. Thus, further experiments which can assess (1) the order of the binding affinity of drugs, (2) the participation or role of the definite function of the tissue and (3) the determination of amino acid sequences of $\alpha_1$-

### Table II. $pK_1$ Values of Terazosin and Its Isomers for $\alpha_{1L}$-Adrenoceptor Subtypes in Various Tissues

<table>
<thead>
<tr>
<th>Drug</th>
<th>Bovine prostate</th>
<th>Canine aorta</th>
<th>Rat brain</th>
<th>Rat heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin</td>
<td>8.34±0.15 (3)</td>
<td>8.45±0.22 (8)</td>
<td>8.04±0.23 (3)</td>
<td>8.87±0.25 (4)</td>
</tr>
<tr>
<td>Benzazosin</td>
<td>8.35±0.23 (3)</td>
<td>8.34±0.19 (6)</td>
<td>9.87±0.10 (5)</td>
<td>8.61±0.11 (4)</td>
</tr>
<tr>
<td>(S−)Terazosin</td>
<td>7.68±0.18 (6)</td>
<td>8.58±0.24 (5)</td>
<td>7.93±0.16 (8)</td>
<td>7.53±0.20 (10)</td>
</tr>
<tr>
<td>(rac)Terazosin</td>
<td>7.61±0.26 (9)</td>
<td>8.50±0.23 (6)</td>
<td>7.78±0.19 (7)</td>
<td>7.69±0.20 (10)</td>
</tr>
<tr>
<td>(R+) Terazosin</td>
<td>6.96±0.15 (6)</td>
<td>8.38±0.09 (5)</td>
<td>7.66±0.16 (8)</td>
<td>7.73±0.15 (8)</td>
</tr>
</tbody>
</table>

Values in parentheses indicate the number of experiments. Data are the mean±S.E.
adrenoceptor structure are needed to clarify the coincidence between $\alpha_{1,\text{High}}$ (or $\alpha_{1,\text{Low}}$) on one tissue and another. The same elucidation between the subtypes used in the present study and the other $\alpha_{1}$-adrenoceptor subtypes reported so far will be also required.

In conclusion, the radioligand binding assay method used in the present study was useful for the assessment of detailed $\alpha_{1}$-blocking potency of terazosin enantiomers. In addition, the highest displacental potency of ($S-$) terazosin may largely contribute to the treatment of hypertension and benign prostatic hyperplasia.

REFERENCES