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α_1-Adrenoceptor Subtypes in Bovine Prostate

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Abstract—The object of this study was to examine the existence and characteristics of α_1-adrenoceptor subtypes in the bovine prostate using the radioligand binding assay method. [^{3}H]Prazosin was used as the radioligand and its binding sites in bovine prostate were classified into two subtypes. One subtype showed a high affinity (α_{1High}, K_d: 101·1 pM and B_{max}: 11·8 fmol (mg protein)$^{-1}$) and the other had a low affinity (α_{1Low}, K_d: 3371·4 pM and B_{max}: 50·5 fmol (mg protein)$^{-1}$). Although the same pK_i values of clorethylclonidine, *p*-aminoclonidine, benoxathian and dibenamine to both α_{1High} and α_{1Low} binding sites in bovine prostate tissue were observed, other α_1 antagonists used in this study had different pK_i values for the two α_1-adrenoceptor subtypes. The existence and binding characteristics of α_1-adrenoceptor subtypes in bovine prostate were clarified. It is possible that agents selective for one site may contribute to the development of better drugs for the treatment of bladder outlet obstructions of men with benign prostatic hyperplasia.

Our previous report (Maruyama et al 1991) described the presence of α_1-adrenoceptors in bovine prostates using radioligand binding assay methods. α_1-Adrenergic antagonists, such as prazosin and phenoxybenzamine, are currently clinically used in the treatment of bladder outlet obstructions of men with benign prostatic hyperplasia (BPH) (Cain et al 1976, 1978; Gerstenberg et al 1980; Shapiro et al 1981; Furuya et al 1982; Hedlund et al 1983; Cain 1986). Thus, α_1-blockers play important roles in the treatment of these diseases.

α_1-Adrenoceptors have been classified into two subtypes by classical pharmacological techniques or radioligand binding assay methods (Flavahan & Vanhoutte 1986; Han et al 1987; McGrath & Wilson 1988; Harrison et al 1991).

This study was designed to investigate details of the existence and characteristics of α_1-adrenoceptors in bovine prostate and to determine the affinity of several drugs for the two subtypes (α_{1High} and α_{1Low}) using the radioligand binding assay method.

Materials and Methods

Materials

[^{3}H]Prazosin (87·0 Ci mmol^{-1}) was purchased from new England Nuclear Corporation, Ltd, Japan, and was stored at −20°C. Prazosin, dibenamine, clonidine (Funakoshi, Japan), WB-4101 (2-[*N*-(2,6-dimethoxyphenoxyethyl)] amino methyl-1,4-benzodioxane; Amersham, Japan), phenoxybenzamine (Nakalai Tesqu, Japan), phentolamine (Ciba-Geigy, Japan), benoxathian, 2[[[2-2, 6-dimethoxyphenoxy-ethyl]amino]methyl]-1,4-benoxathian hydrochloride hydrate, benextramine, *N,N*-(dithiodi-2,1-ethanediyl)bis[*N'*-(2-methoxyphenyl)methyl]1,6-hexanediamine tetrahydrochloride monohydrate, chlorethylclonidine, *p*-aminoclonidine (Research Biochemicals, USA), yohimbine (Nakarai Chemicals, Japan), labetalol (ICI Pharma, Japan), 5-hydroxytryptamine (Wako Pure Chemical Industries, Japan) were purchased. Bunazosin (Eizai, Japan), SGB-1534, 3-[2-[4-*o*-methoxyphenyl)-1-piperazinyl]-ethyl]-2,4-(1H,3H)-quinazolinedione monohydrochloride (Chugai, Japan), HV-723 (α-ethyl-3,4,5-trimethoxy-α(3((2-(2-methoxy-phenoxy)ethyl)-amino)-propyl) benzenecontonitrile fumerate; Hokuriku Seiyaku, Japan), Terazosin (Mitsubishi Kasei, Japan), amosulalol, (5-[1-hydroxy-2-[[2-(*o*-methoxyphenoxy)ethyl]amino]ethyl]2-methyl-benzenesulphonamide hydrochloride (Yamanouchi Pharma, Japan), arotinolol, nifedipine (Sumitomo Chemical, Japan), ketanserin (Kyowa Hakko Kogyo, Japan), cinanserin (E. R. Squibb & Sons, USA), chlorpromazine (Yoshitomi Pharma, Japan) were kindly donated by each company. All compounds used in the present study (Table 2), except for nifedipine, were diluted with distilled water. Nifedipine was dissolved in ethanol and diluted appropriately with distilled water. All prepared compounds were stored at 4°C.

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Preparation of a crude membrane-enriched fraction from the bovine prostate

Crude membranes were prepared by the method described previously except that the incubation buffer did not contain $MgCl_2$ (Maruyama et al 1991). In brief, the prostate (3–5 g) was removed, frozen in liquid nitrogen and stored at −80°C until used. The prostate tissue was defrosted at room temperature (21°C) and minced with scissors in 10 vol of 50 mM Tris–HCl buffer containing 10 mM $MgCl_2$, pH 7·4 at 4°C. All procedures for preparation of the crude membrane-enriched fraction were performed at 4°C. The suspension was homogenized with a Polytron homogenizer, 5–10 times for 10 s at setting 8, then filtered through 4 layers of gauze. The filtrate was centrifuged for 5 min at 100 *g*, and the supernatant was again centrifuged for 30 min at 40 000 *g*. The pellet was rinsed once with the incubation buffer (120 mM Tris–HCl, pH 7·4) and homogenized using a glass homogenizer in the same buffer. The fraction was then immediately frozen in liquid nitrogen and stored at −80°C until use. Protein concentration was determined by the method of Lowry et al (1951), using bovine serum albumin as the standard.

Binding assay

α_1-Adrenoceptors in bovine prostate were examined using [^{3}H]prazosin as the radioligand. The saturation experiments were performed in the range of 0·1–5·0 nM [^{3}H]prazosin, and the displacement experiments were carried out using 0·12 ($\alpha_{1\,High}$) and 0·5 nM ($\alpha_{1\,Low}$) [^{3}H]prazosin. In the binding assay for $\alpha_{1\,Low}$ receptors, 10^{-9} M bunazosin was present in a membrane suspension. A membrane suspension (0·2 mg of protein) containing [^{3}H]prazosin and various α_1-antagonists in 0·5 mL of 60 mM Tris–HCl buffer, pH 7·4, was incubated at 23°C for 30 min, and terminated by rapid filtration under vacuum through glass fibre filters (Whatman GF/C) using an Automatic Cell Harvester Labomash (LM-101, Labo Science). The filters were added to 1 mL of a toluene–triton based scintillation fluid and the radioactivity was counted by scintillation spectrometry (Packard 2200 Tri-Carb Scintillation Analyzer). The specific binding of [^{3}H]prazosin was defined as the difference between the total binding and the nonspecific binding in the presence of 10 μM phentolamine. Inhibition constants (K_i) were calculated by the method previously described (Tsuchihashi & Nagatomo 1987) and expressed as pK_i ($-\log K_i$).

Results

Fig. 1a,b shows typical results of the saturation experiments and the Scatchard analysis, respectively. These two diagrams suggest that [^{3}H]prazosin binds to both subtypes of α_1-adrenoceptors as the specific binding curve in Fig. 1a is shown to change near 0·7 nM [^{3}H]prazosin, and the Hill coefficient (Fig. 1b) was 0·54. Fig. 1c depicts the results of the computer analysis of results in Fig. 1b. These two lines represent the curve of Fig. 1b and show the two binding sites, $\alpha_{1\,High}$ having high affinity and $\alpha_{1\,Low}$ having low affinity for [^{3}H]prazosin. Fig. 1d shows typical Scatchard plots of the saturation experiments carried out in the presence of 10^{-9} M bunazosin. This linearity indicated it was possible to analyse only $\alpha_{1\,Low}$ binding sites as 10^{-9} M bunazosin could completely displace [^{3}H]prazosin bindng at $\alpha_{1\,High}$-sites. No differences in values of B_{max} and K_d for $\alpha_{1\,Low}$-receptors were obtained in the absence and presence of 10^{-9} M bunazosin (Table 1). Thus, this assay was thereafter applied to the determination of the $\alpha_{1\,Low}$-binding sites.

Table 1 summarizes values of the dissociation constants (K_d) and the maximum number of binding sites (B_{max}) of $\alpha_{1\,High}$- and $\alpha_{1\,Low}$-binding sites in bovine prostate tissue for

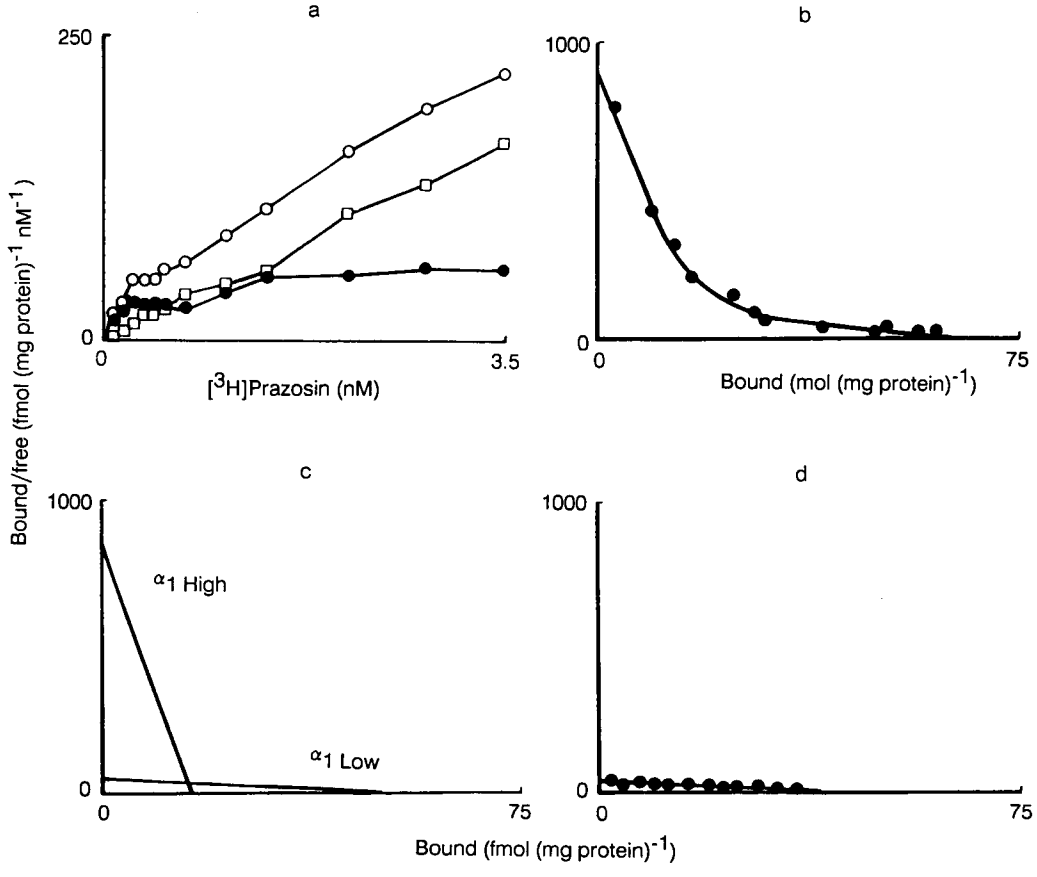

FIG. 1. Binding characteristics of [^{3}H]prazosin to α_1-adrenoceptor subtypes in bovine prostate. a. Saturation curve of a typical experiment. The specific binding of [^{3}H]prazosin (●) was defined as the difference between the total binding (○) and nonspecific binding in the presence of 10 μM phentolamine (□). b. Scatchard plot obtained from a. c. Computer analysis of b. $\alpha_{1\,High}$; K_d 101·06 pM, B_{max} 11·76 fmol (mg protein)$^{-1}$. $\alpha_{1\,Low}$; K_d 3371·38 pM, B_{max} 50·45 fmol (mg protein)$^{-1}$. Hill coefficient; 0·54. d. Scatchard plot in the presence of 10^{-9} M bunazosin.

Table 1. Binding characteristics of [^{3}H]prazosin for α_1-adrenoceptor subtypes in bovine prostate.

$\alpha_{1\,High}$-Affinity site		$\alpha_{1\,Low}$-Affinity site	
K_d (pM)	B_{max} fmol (mg protein)$^{-1}$	K_d (pM)	B_{max} fmol (mg protein)$^{-1}$
101·06 ± 65·02	11·76 ± 2·32	3371·38 ± 892·15	50·45 ± 2·65 (4)
—	0	1132·39 ± 67·26	48·70 ± 8·94 (3)

Values in parentheses indicate the number of experiments. Data are the mean ± s.e. Each value in the lower rows was obtained from the experiments in the presence of 10^{-9} M bunazosin.

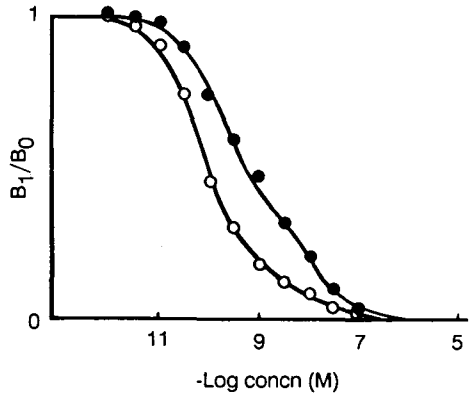

FIG. 2. Displacement curves of bunazosin using 0·5 (●) and 0·12 nM (○) of [^{3}H]prazosin.

[^{3}H]prazosin. Data were analysed using Scatchard analysis. When 10^{-9} M bunazosin was used in the binding assay for Scatchard analysis, $\alpha_{1\,High}$-binding sites disappeared (Fig. 1c) and the values of K_d and B_{max} of $\alpha_{1\,Low}$-binding sites obtained from non-linear Scatchard analysis coincided with those obtained in the presence of 10^{-9} M bunazosin (Table 1).

A displacement experiment was performed to evaluate the displacement potencies (pK_i values) of various drugs to each α_1-adrenoceptor subtype. Typical dose–displacement curves for bunazosin are shown in Fig. 2. Different concentrations of [^{3}H]prazosin were used for the analysis; the displacement curve for which a high concentration of [^{3}H]prazosin (0·5 nM) was used is shallow, implying that it contains two binding sites. On the other hand, the curve for which a low concentration (0·12 nM) of this radioligand was used shows a monophasic curve. Computer analysis of the results with high [^{3}H]prazosin concentration showed that the turning point of the curve represented the presence of the $\alpha_{1\,High}$- and $\alpha_{1\,Low}$-binding sites, and the concentration at this change was 10^{-9} M bunazosin. When we used this concentration (10^{-9} M) in the incubation medium, the $\alpha_{1\,High}$-binding site disappeared. The ratio of $\alpha_{1\,High}/\alpha_{1\,Low}$ was shown by calculation to be 90% (Tsuchihashi & Nagatomo 1987; Tsuchihashi et al 1989a, b), and this curve (the low concentration of [^{3}H]prazosin) gives us only $\alpha_{1\,High}$ pK_i value of bunazosin.

Based on the data found in Fig. 2, the two displacement curves of prazosin shown in Fig. 3 were obtained. Results using the high (0·5 nM) concentration of [^{3}H]prazosin in the

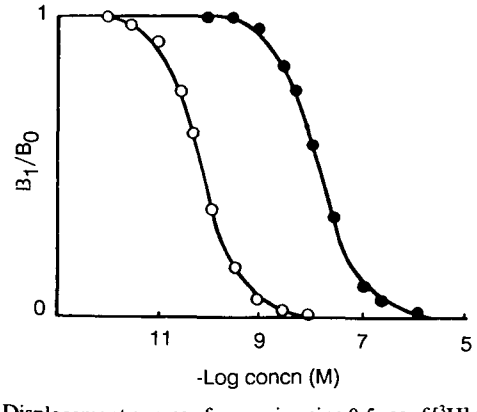

FIG. 3. Displacement curves of prazosin using 0·5 nM of [^{3}H]prazosin in the presence ($\alpha_{1\,Low}$) (●) and 0·12 nM of [^{3}H]prazosin in the absence ($\alpha_{1\,High}$) (○) of 10^{-9} M bunazosin.

Table 2. The pK_i values of various drugs for α_1-adrenoceptor subtypes in bovine prostate.

Drugs	$\alpha_{1\,High}$-Affinity site	$\alpha_{1\,Low}$-Affinity site
α_1-Adrenoceptor-related agents		
Prazosin***	10·64 ± 0·16 (3)	8·34 ± 0·15 (3)
WB-4101***	10·64 ± 0·12 (5)	8·32 ± 0·20 (3)
Bunazosin***	9·87 ± 0·24 (4)	8·35 ± 0·23 (3)
HV-723***	9·73 ± 0·19 (4)	7·59 ± 0·16 (3)
SGB-1534***	9·56 ± 0·11 (3)	8·15 ± 0·08 (3)
Phenoxybenzamine***	9·30 ± 0·20 (3)	7·97 ± 0·08 (3)
Terazosin***	9·17 ± 0·17 (5)	7·54 ± 0·21 (3)
Amosulalol***	8·96 ± 0·06 (3)	7·05 ± 0·21 (3)
Phentolamine***	8·68 ± 0·13 (5)	5·56 ± 0·06 (3)
Benoxathian	7·99 ± 0·20 (3)	7·67 ± 0·12 (3)
Benextramine*	6·97 ± 0·13 (3)	6·24 ± 0·20 (3)
Dibenamine	5·64 ± 0·05 (3)	5·67 ± 0·06 (3)
α_2-Adrenoceptor-related agents		
Labetalol***	7·95 ± 0·24 (3)	6·39 ± 0·14 (4)
Yohimbine**	7·55 ± 0·16 (4)	6·78 ± 0·08 (3)
p-Aminoclonidine	6·64 ± 0·02 (3)	6·55 ± 0·05 (3)
Clonidine**	6·39 ± 0·02 (3)	5·73 ± 0·16 (3)
Chlorethylclonidine	5·68 ± 0·14 (3)	5·13 ± 0·19 (3)
β-Adrenoceptor-related agent		
Arotinolol***	6·65 ± 0·17 (3)	5·62 ± 0·12 (3)
5-HT-receptor-related agents		
Ketanserin*	8·31 ± 0·09 (3)	7·76 ± 0·16 (3)
Cinanserin	6·22 ± 0·13 (3)	6·41 ± 0·22 (6)
5-Hydroxytryptamine*	4·76 ± 0·25 (5)	3·85 ± 0·07 (3)
Ca^{2+} receptor-related agent		
Nifedipine	5·43 ± 0·12 (3)	4·94 ± 0·17 (3)
Psychotropic-related agent		
Chlorpromazine***	9·54 ± 0·20 (3)	6·43 ± 0·27 (3)

Values in parentheses indicate the number of experiments. Data are the mean ± s.e. *** $P < 0·01$, ** $P < 0·02$, * $P < 0·05$ in comparing both types of receptor.

presence of 10^{-9} M bunazosin show pK_i values which indicate that the $\alpha_{1\,Low}$ binding study and the displacement study using low (0·12 nM) [^{3}H]prazosin concentration in the absence of bunazosin, show the $\alpha_{1\,High}$ pK_i value of prazosin. No difference in the K_i or K_d value of prazosin was found in either the displacement experiment or Scatchard analysis.

Table 2 summarizes the pK_i values of various drugs for each α_1-adrenoceptor subtype.

Discussion

Most agents used in this study were able to distinguish between $\alpha_{1\,High}$- and $\alpha_{1\,Low}$-binding sites in bovine prostate tissue with the exception of benoxathian, dibenamine, chlorethylclonidine, *p*-aminoclonidine, cinanserin and nifedipine (Table 2). In spite of the similar structures of benoxathian and WB-4101, only WB-4101 could distinguish between $\alpha_{1\,High}$- and $\alpha_{1\,Low}$-binding sites.

When we compared the structure of benoxathian with that of WB-4101, the benzo-1,4-xathian residue in benoxathian displaced the benzo-1,4-dioxan in WB-4101. The only difference between these residues is in the atom at the 4-position of the benzo-binding six-membered ring which is sulphur for benoxathian or oxygen for WB-4101, thus suggesting that these different atoms could alter the affinity of the compound for each subtype. Thus, since the oxygen atom has higher electronegativity than the sulphur atom, this stronger electron affinity may play a role in binding.

In addition, the oxygen atom in the long side-chain of phenoxybenzamine may have the same important function as that in the structure of WB-4101 in the drug-receptor interaction.

Chlorethylclonidine and *p*-aminoclonidine could not differentiate the two α_1-adrenoceptor subtypes although clonidine could. Thus the residues bound to clonidine appear to have a negative influence on the ability to recognize $\alpha_{1\,High}$- and $\alpha_{1\,Low}$-binding sites or their environs.

With respect to the prazosin and terazosin used in this study, prazosin was found, from its pK_i value, to have the most clear effect in differentiating the subtypes (Maruyama et al 1991). The chemical structures of these two agents differ in that prazosin has a furan residue and terazosin contains a tetrahydrofuran residue. The presence or absence of the double bond may effect the drug-receptor interaction in the bovine prostate.

Coates et al (1982) suggested that the α_1-adrenoceptor is subdivided into at least two subtypes, and that one subtype (α_{1S}) is characterized by high sensitivity to Sgd 101/75 (4(2-imidazoline-amino)-2-methylin-dazol-chlorhydrate) and phenoxybenzamine. They also suggested that this receptor, which is blocked by a very low concentration of phenoxybenzamine, was involved in strong contraction of the prostate; however, it is not yet known which subtype ($\alpha_{1\,High}$ or $\alpha_{1\,Low}$) participates in this contraction. The pA_2 values of several drugs reported in other papers (Nagatomo et al 1985; Tsuchihashi & Nagatomo 1989) appear to correlate with the pK_i values of $\alpha_{1\,High}$-binding sites obtained in this paper but not with the $\alpha_{1\,Low}$-adrenoceptors. Thus, further studies are needed to evaluate the physiological function of these two subtypes in the prostate.

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