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α_1-Adrenoceptor Subtypes in the Rat Ventricular Muscle

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Abstract—Scatchard analyses of [^{3}H]prazosin binding in rat ventricular muscle membranes showed biphasic curves, which identified α_{1High}- and α_{1Low}-affinity sites. The α_{1High}-affinity site was completely inhibited by 1 μM phenoxybenzamine. The displacement potencies of α_1-adrenergic antagonists were characterized by [^{3}H]prazosin binding to α_{1High}- and α_{1Low}-affinity sites in the absence and presence of 1 μM phenoxybenzamine. The affinities of most chemicals for α_{1Low}-affinity sites were significantly lower than those for α_{1High}-affinity sites, but WB-4101 (2-(2,6-dimethoxy-phenoxyethyl)aminomethyl-1,4-benzodioxane), arotinolol, cinanserin, nifedipine, and *p*-aminoclonidine had the same affinities for both α_{1Low}- and α_{1High}-affinity sites. These results show that two α_1-adrenoceptor subtypes, α_{1High}- and α_{1Low}-affinity, are present in the rat heart, and that there are physical variations in α_1-adrenoceptor binding sites, based on their selectivity to antagonists.

There is increasing evidence that the heterogeneity of α_1-adrenoceptors is related to the existence of pharmacologically distinct subtypes. Coates et al (1982) noted that two distinct subtypes in the rat anococcygeum had different affinities for phenoxybenzamine. Flavahan & Vanhoutte (1986) suggested that there were two distinct subtypes, α_{1H} and α_{1L}, which could be distinguished by their affinities for both prazosin and yohimbine in blood vessels. Radioligand binding studies with [^{3}H]prazosin demonstrated two subtypes in the rat brain, of which the α_{1a}-subtypes had a higher affinity for WB-4101 than the α_{1b}-subtypes (Morrow & Creese 1986). Han et al (1987a, b) also reported that two distinct subtypes, α_{1A} and α_{1B}, were distinguished by ^{125}I-BE 2254 (iodo-2-[β-(4-hydroxyphenyl)-ethyl-aminomethyl]tetralone) binding in various rat tissues. This distinction was made on the basis of the affinities of WB-4101 and benoxathian and of sensitivity to chlorethylclonidine and the organic Ca^{2+} channel blocker, nifedipine. Muramatsu et al (1990) suggested that there were three distinct subtypes in the blood vessels, α_{1H}, α_{1L}, and α_{1N}. This distinction was made on the basis of the antagonistic potencies of phentolamine, yohimbine, WB-4101, prazosin, and HV-723(α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy)-ethyl)-amino)-propyl)-benzeneacetonitrile fumarate) and of the sensitivity to chlorethylclonidine and nifedipine. A subclassification has been proposed for ^{125}I-BE 2254 binding sites, using bovine and hamster clone cDNA, of the α_1-adrenoceptor expressed in COS7 cells (Schwinn et al 1990).

We also reported that a binding assay using [^{3}H]prazosin as the radioligand and rat brain and canine aorta as sources of α_1-adrenoceptors was useful in assessing the affinities of α_1-adrenergic antagonists and that most of their affinities correlated with antagonistic potency in the rat aorta (Nagatomo et al 1985; Tsuchihashi & Nagatomo 1989).

It has recently been shown that positive inotropic effects are mediated by cardiac α_1-adrenoceptors in rat (Goto et al 1987; Otani et al 1988), guinea-pig (Goto et al 1987), bovine (Bruckner & Scholz 1984), and rabbit heart (Endoh & Blinks 1988; Hiramoto et al 1988). The purposes of the present study were to examine the characteristics of [^{3}H]prazosin binding to α_1-adrenoceptors in the rat heart and to assess the affinities of various ligands.

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Materials and Methods

Materials and animals

[^{3}H]Prazosin (76·6 Ci mmol^{-1}) was purchased from New England Nuclear/Dupont Ltd, Boston, MA, USA. HV-723, α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy)-ethyl)-amino)-propyl)-benzenacetonitrile fumarate, was kindly donated by Hokuriku Seiyaki (Katsuyama, Fukui, Japan). Male Wistar rats, 250–350 g, were killed by decapitation after CO_2 asphyxiation.

Preparation of membrane-enriched fraction

After the rat heart was removed the ventricular muscles were immediately frozen in liquid nitrogen and stored at −80°C until use. Membrane-enriched fractions from the rat ventricular muscle were prepared as follows. Tissues were defrosted at room temperature (21°C) and minced with scissors in 10 vol of buffer I (0·25 M sucrose, 10 mM Tris-HCl buffer, pH 7·4). The suspensions were homogenized, using a Polytron homogenizer, twice for 10 s at setting 8. Homogenates were filtered through 4 layers of gauze. The filtrate was centrifuged at 40 000 *g* for 30 min. The pellet was rinsed at once and homogenized in buffer II (120 mM Tris-HCl, pH 7·4), using a glass homogenizer. The membrane-enriched fraction was frozen in liquid nitrogen, stored at −80°C, and diluted to appropriate concentrations immediately before use. There was no observable quantitative or qualitative decrease in binding of the membrane-enriched fraction after two months storage compared with the fraction which was freshly prepared without being thawed. Protein was determined using the method of Lowry et al (1951).

Binding assay

The membrane suspension (0·1 mg of protein) was incubated for 45 min at 23°C in a total volume of 0·5 mL containing 60 mM Tris-HCl (pH 7·4). In the saturation experiments thirteen [^{3}H]prazosin concentrations were used, in the range of 0·1–5·0 nM. In the displacement experiments, the [^{3}H]prazosin

concentrations were 0·1 and 0·6 nM. The affinity of the α_{1Low}-subtype for ligands was determined in the presence of 1 μM of phenoxybenzamine which inhibited the α_{1High}-subtype completely. At the end of the incubation period, the incubation medium was immediately filtered through GF/C glass fibre and was washed with incubation buffer using the method described previously (Tsuchihashi et al 1985). The radioactivity of the filter was counted with a Packard 2200 Tri-Carb Scintillation Analyzer. The specific binding was determined by subtracting the non-specific binding in the presence of 10 μM of phentolamine from the total.

Kinetic analysis

All kinetic analyses were carried out on an NEC PC-9801 computer system using an iterative non-linear regression program (Tsuchihashi & Nagatomo 1987; Tsuchihashi et al 1989a, b, 1990). The fits of a model having only one receptor subtype and a model with two were compared (Tsuchihashi & Nagatomo 1987; Tsuchihashi et al 1989a, b, 1990). Most K_i values of various ligands are expressed as pK_i ($-\log K_i$) in this report. In order to quantify the mode of saturation and the displacement character, Hill numbers for Scatchard analysis and slope factor (n_H) for displacement curves were determined as described previously (Tsuchihashi & Nagatomo 1987; Tsuchihashi et al 1989a, b, 1990).

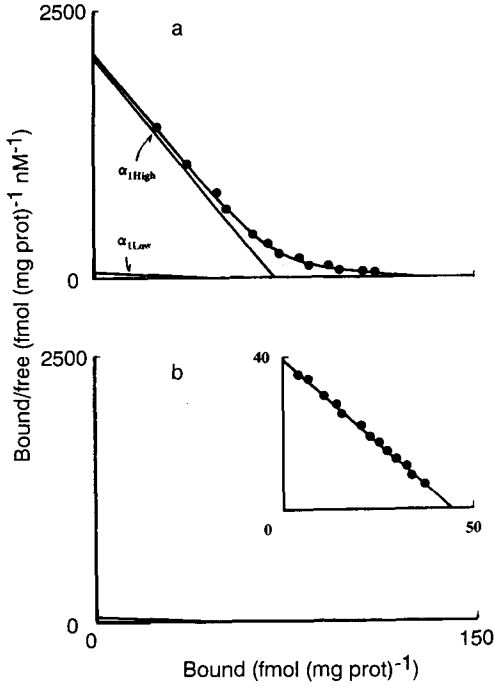

FIG. 1. Scatchard plots of [^{3}H]prazosin binding to rat ventricular muscle in the absence (a) and presence (b) of 1 μM phenoxybenzamine. Specific binding is defined as the difference between the total binding and the binding in the presence of 10 μM phentolamine. (a) Scatchard plot and data after computer analysis in the absence of phenoxybenzamine: α_{1High}-subtypes; K_d, 35·35 pM; B_{max}, 72·58 fmol (mg prot.)$^{-1}$; α_{1Low}-subtypes; K_d, 2050·94 pM; B_{max}, 61·12 fmol (mg prot.)$^{-1}$, Hill number; 0·72. (b) Scatchard plot in the presence of phenoxybenzamine: K_d, 1107·76 pM; B_{max}, 44·13 fmol (mg prot.)$^{-1}$, Hill number; 1·01. The data shown are those from a single experiment performed in duplicate.

Results

Fig. 1 shows the Scatchard plots of the [^{3}H]prazosin binding data from rat ventricular muscle. Whereas two binding sites, α_{1High}- and α_{1Low}-affinity sites, emerged in the absence of 1 μM phenoxybenzamine, only one subtype, α_{1Low}, was found in the presence of 1 μM phenoxybenzamine. The K_d and B_{max} values in the presence of phenoxybenzamine were identical to those of the α_{1Low}-affinity site of the two subtypes (Table 1), thus suggesting that the α_{1High}-affinity site was completely inhibited by 1 μM phenoxybenzamine.

Fig. 2 shows the displacement curves of phenoxybenzamine and prazosin in the absence (a,b) and presence (b) of 1 μM phenoxybenzamine. The displacement curves of phenoxybenzamine used as competitor were biphasic at both 0·1 and 0·6 nM of [^{3}H]prazosin (a). When the radioligand concentration was increased from 0·1 to 0·6 nM, the apparent proportional percentages of α_{1High}-affinity sites for phenoxybenzamine decreased from 92·5 to 75·9%, whereas those of α_{1Low}-affinity sites increased from 7·5 to 24·11% (Fig. 2a). When displacement assays were performed in the presence of 1 μM of phenoxybenzamine and 0·6 nM [^{3}H]prazosin, the displacement curves were shifted toward the right and were monophasic (Fig. 2b). Thus, we decided that the optimal concentrations of [^{3}H]prazosin required to determine the affinities of competitors were 0·1 and 0·6 nM for the α_{1High}- and α_{1Low}-affinity subtypes, respectively. Only 7·5% of the α_{1Low}-subtypes were revealed by the displacement curves (Fig. 2a) and the slope factor was close to 1·0 (0·94, 0·78 in the case of 0·6 nM) at 0·1 nM [^{3}H]prazosin (Fig. 2b). Affinities of phenoxybenzamine and prazosin for α_{1High}-subtypes (Fig. 2b) were not observed at 0·6 nM of [^{3}H]prazosin in the presence of 1 μM phenoxybenzamine.

Table 2 shows the pK_i values of various α_1-adrenoceptor antagonists and α_1-adrenoceptor-related agents for two kinds of α_1-adrenoceptor subtypes. The pK_i values of most agents for the α_{1Low}-subtype were significantly lower than those for the α_{1High}-subtype, whereas WB-4101, arotinolol, nifedipine, cinanserin, and *p*-aminoclonidine had the same pK_i values for both α_{1High}- and α_{1Low}-subtypes in the heart.

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

		α_{1Low}-Affinity site		α_{1High}-Affinity site	
		K_d (pM)	B_{max} (fmol (mg prot.)$^{-1}$)	K_d (pM)	B_{max} (fmol (mg prot.)$^{-1}$)
Rat heart with phenoxybenzamine	(3)	1609·67 ± 229·06	57·66 ± 2·44	32·39 ± 10·33	71·18 ± 4·27
	(3)	1296·54 ± 94·84	51·05 ± 12·86	—	0

Parentheses indicate the number of experiments. Data are mean values ± s.e.

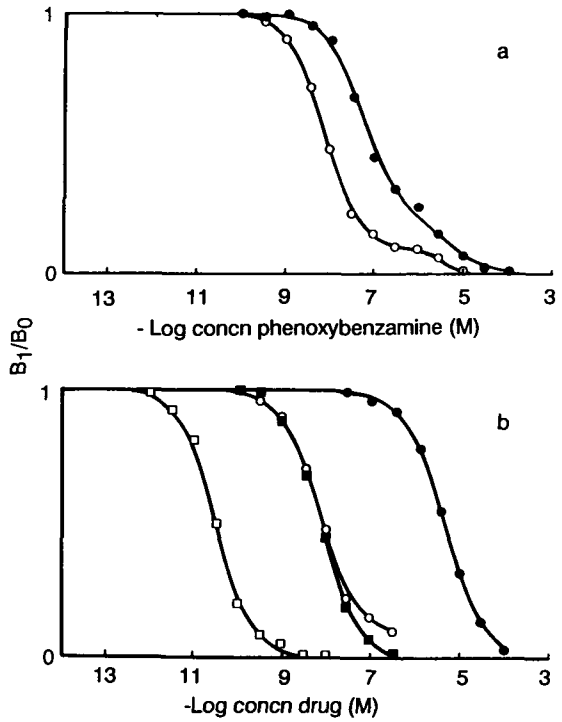

FIG. 2. Displacement by phenoxybenzamine (a, b) and prazosin (b) for [^{3}H]prazosin binding in the rat ventricular muscle. (a) Displacement curves of phenoxybenzamine at 0·1 nM (○) and 0·6 nM [^{3}H]prazosin (●). The apparent proportional percentages of α_{1High}- and α_{1Low}-affinity sites were 92·5 and 7·5% at 0·1 nM [^{3}H]prazosin (slope factor = 0·82) and 75·9 and 24·1% at 0·6 nM [^{3}H]prazosin (slope factor = 0·76). (b) Displacement curves of prazosin (□ or ■) and phenoxybenzamine (○ or ●) in the absence (○ or □) of 1 μM phenoxybenzamine at 0·1 nM [^{3}H]prazosin and presence (● or ■) of 1 μM phenoxybenzamine at 0·6 nM [^{3}H]prazosin. The slope factors of prazosin and phenoxybenzamine in b were close to 1·0 in the absence and presence of 1 μM phenoxybenzamine, except for those of phenoxybenzamine (0·94) in the absence of 1 μM phenoxybenzamine. B_1 and B_0 are the concentrations of the radioligand bound with or without the unlabelled ligand, respectively. The data shown are those from a single experiment performed in duplicate.

Table 2. pKi values of α_1-adrenoceptor antagonists for α_{1Low}- and α_{1High}-subtypes.

	[α_{1Low}]	[α_{1High}]
Prazosin	8·87 ± 0·25 (4)	11·14 ± 0·04 (4)****
HV-723	7·21 ± 0·18 (3)	8·93 ± 0·02 (3)***
WB-4101	7·66 ± 0·27 (4)	8·50 ± 0·18 (3)
Bunazosin	8·61 ± 0·11 (4)	9·95 ± 0·03 (3)****
Terazosin	7·90 ± 0·10 (4)	9·16 ± 0·04 (3)****
Phenoxybenzamine	5·98 ± 0·17 (4)	9·27 ± 0·23 (3)****
Phentolamine	5·30 ± 0·20 (3)	7·68 ± 0·20 (3)****
Ketanserin	7·57 ± 0·12 (3)	8·28 ± 0·08 (4)***
SGB-1534	8·56 ± 0·12 (3)	9·25 ± 0·18 (3)*
Chlorpromazine	7·02 ± 0·02 (3)	9·04 ± 0·08 (3)****
Benoxathian	6·95 ± 0·14 (4)	8·44 ± 0·18 (4)****
Benextramine	6·83 ± 0·15 (3)	7·54 ± 0·11 (3)**
YM-09538	6·46 ± 0·16 (3)	7·85 ± 0·17 (3)***
Labetalol	5·85 ± 0·09 (3)	6·89 ± 0·05 (3)****
Yohimbine	5·87 ± 0·17 (4)	6·58 ± 0·09 (3)**
Dibenamine	4·93 ± 0·07 (3)	5·89 ± 0·11 (3)***
Arotinolol	5·97 ± 0·27 (4)	6·04 ± 0·23 (3)
Clonidine	4·66 ± 0·21 (3)	6·15 ± 0·16 (3)***
Chlorethylclonidine	5·21 ± 0·06 (3)	6·03 ± 0·10 (4)***
Cinanserin	6·06 ± 0·13 (3)	6·01 ± 0·18 (3)
Nifedipine	5·67 ± 0·17 (3)	5·38 ± 0·02 (3)
5-HT	3·84 ± 0·10 (3)	4·42 ± 0·03 (3)***
p-Aminoclonidine	7·31 ± 0·34 (3)	6·61 ± 0·14 (3)

Parentheses indicate the number of experiments. Data are mean values ± s.e. *$P < 0·05$, **$P < 0·02$, ***$P < 0·01$, ****$P < 0·001$, vs α_{1Low}.

Discussion

Scatchard analysis of binding data in the rat ventricular muscles revealed curves that were biphasic in the absence of 1 μM of phenoxybenzamine and monophasic in its presence. The values of K_d and B_{max} in the presence of phenoxybenzamine were identical with those of the α_{1Low}-subtype of the two subtypes in the absence of phenoxybenzamine. These subtypes, α_{1High} and α_{1Low}, were distinguished by their affinity for prazosin. We also found that these two subtypes, had different affinities for α_1-adrenoceptor-related agents, thus suggesting a physical variation, based on antagonistic selectivity, in α_1-adrenoceptor binding sites.

Flavahan & Vanhoutte (1986) suggested that there were two distinct subtypes, α_{1H} and α_{1L}, which could be distinguished by their affinities for both prazosin and yohimbine in blood vessels. Prazosin and yohimbine were more selective to α_{1High}- than to α_{1Low}-subtypes in the present study. Coates et al (1982) found that two distinct subtypes in the rat anococcygeus had different affinities for phenoxybenzamine. A low concentration of phenoxybenzamine (0·3 nM) antagonized the high-affinity site of α_1-adrenoceptors in the rat anococcygeum. The α_{1High}-subtype also had high affinity for phenoxybenzamine ($pK_i = 9·27$) in the present study. Radioligand binding studies with [^{3}H]prazosin demonstrated two subtypes in the rat brain, of which α_{1a}-subtypes had a higher affinity for WB-4101 than α_{1b}-subtypes (Morrow & Creese 1986). However, WB-4101 was not significantly selective towards either α_{1High}- or α_{1Low}-subtypes in the present study.

Han et al (1987a) showed that two distinct subtypes, α_{1A} and α_{1B}, were distinguished by ^{125}I-BE 2254 binding in the rat hippocampus and vas deferens. This distinction was made on the basis of the affinities of WB-4101 and benoxathian and of the sensitivity to chlorethylclonidine and the organic Ca^{2+} channel blocker nifedipine. Han et al (1987a) also suggested that only one subtype (α_{1B}) existed in rat liver and spleen. Compared with the α_{1A}-subtype, the α_{1B}-subtype was more sensitive to chlorethylclonidine and less sensitive to WB-4101, benoxathian, and nifedipine. The similar results obtained in this study suggested that α_{1High}- and α_{1Low}-subtypes were similar to α_{1A}- and α_{1B}-subtypes, respectively. In this study, chlorethylclonidine and nifedipine were dealt with as competitive antagonists, although they are known as non-competitive. It is known that β-haloalkylamines such as phenoxybenzamine, dibenamine and chlorethylclonidine behave as competitive antagonists in the early stage (competitive stage) and then allow covalent chemical bonding of the drugs to some grouping at its site of action in the receptor (non-competitive stage). Nifedipine is a 1,4-dihydropyridine compound and a Ca^{2+} channel blocker. Recently, it was found that another 1,4-dihydropyridine Ca^{2+} channel blocker, (+)-nigludipine, was a competitive and sensitive antagonist for α_{1A}-subtypes (Boer et al 1989; Graziadei et al 1989). Chlorethylclonidine and nifedipine are likely to antagonize α_1-adrenoceptors competitively. The slope factors for all these agents in inhibiting specific [^{3}H]prazosin binding were close to 1·0 in the α_{1High}- and α_{1Low}-subtypes,

suggesting a homogeneous population of binding sites in this tissue and behaviour of chlorethylclonidine and nifedipine as competitive antagonists in this assay.

Han et al (1987b), using rat hippocampus membranes (α_{1A}) also showed that the pK_i values of prazosin, WB-4101, phentolamine, benoxathian and yohimbine for ^{125}I-BE 2254 binding were 9·20, 8·93, 7·48, 8·56, and 6·21. The values for prazosin were much less than those (11·14) in that study (α_{1High}), but similar values were observed for WB-4101 (8·50), phentolamine (7·68), benoxathian (8·44), and yohimbine (6·58) in the present study (α_{1High}). Han et al (1987b) also showed that the pK_i values of prazosin, WB-4101, phentolamine, benoxathian and yohimbine in rat vas deferens (α_{1B}) were 9·08, 8·13, 6·97, 7·73, and 6·20, respectively. Their values are similar to those in this study (α_{1Low}): prazosin (8·87); WB-4101 (7·66); and yohimbine (5·87), except for phentolamine (5·30) and benoxathian (6·95).

Muramatsu et al (1990) also suggested that three distinct subtypes existed in the blood vessels, α_{1H} (prazosin (>9·5) > HV-723, WB-4101 (8·0–9·3) > yohimbine (>6·5)), α_{1L} (prazosin, HV-723, WB-4101 (8·0–9·0) > (6·4>) yohimbine), and α_{1N} (HV-723 (9·0>) > WB-4101 (8·4>) > prazosin (8·3>) > yohimbine (>6·5). This distinction was made on the basis of the antagonistic potencies of phentolamine, yohimbine, WB-4101, prazosin, and HV-723 and of the sensitivity to chlorethylclonidine and nifedipine. In our results, the α_{1High}- and α_{1Low}-subtypes were similar to those authors' α_{1H}- and α_{1L}-subtypes, respectively.

A subclassification has been proposed for ^{125}I-BE 2254 binding sites, using bovine and hamster cloned cDNA, of α_1-adrenoceptors expressed in COS7 cells (Schwinn et al 1990). The affinities of two subtypes for prazosin, WB-4101, phentolamine, and yohimbine were 0·26, 8·5, 155, and 1300 nM and 0·27, 0·55, 4·8, and 320 nM in the hamster and bovine α_1-adrenoceptor subtypes, respectively. Hamster and bovine subtypes were respectively similar to α_{1Low}- and α_{1High}-subtypes in this study.

Acknowledgements

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