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α_1-Adrenoceptors: the ability of various agonists and antagonists to discriminate between two distinct [^{3}H]prazosin binding sites

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Abstract—Recently, it has been demonstrated that two distinct α_1-adrenoceptor binding sites showing high and low affinity for WB-4101 (2-(2,6-dimethoxyphenoxy)ethyl-aminomethyl-1,4-benzodioxane) and 5-methyl-urapidil can be distinguished. In the present study we examined the ability of several agonists and antagonists to discriminate between these α_1-adrenoceptor binding sites. [^{3}H]Prazosin binding to membranes of rat liver, heart, cerebral cortex and hippocampus was inhibited monophasically by butanserine, I-BE 2254 (2-(3-(4-hydroxy-3-iodophenyl)ethylaminomethyl)tetralone-hydrochloride), prazosin, rauwolscine and verapamil. In contrast, competition curves of adrenaline, oxymetazoline, amidephrine and YM-12617 (5-[2-[[2-(o-ethoxy-phenoxy)ethyl]-amino]propyl]-2-methoxybenzenesulfonamide HCl)were best described by a model of two binding sites. Chloroethylclonidine (CEC), a compound shown to irreversibly eliminate binding sites with low affinity for WB-4101, increased the proportion of high affinity binding sites for oxymetazoline and amidephrine, whereas the binding data for prazosin and adrenaline remained unchanged. These results indicate that amidephrine, oxymetazoline and YM-12617, but not the other drugs tested discriminate between different α_1-adrenoceptor recognition sites labelled by [^{3}H]prazosin.

The existence of subtypes of the α_1-adrenoceptor remains a matter of controversy (for review see Hieble et al 1987 and McGrath & Wilson 1988). Radioligand binding studies clearly demonstrate that α_1-adrenoceptor recognition sites labelled by [^{3}H]prazosin and [^{125}I]I-BE 2254 (2-(3-(4-hydroxy-3-iodophenyl)ethylaminomethyl)tetralonehydrochloride) are heterogeneous. The sites with high affinity for WB-4101 (2-(*N*-(2,6-dimethoxyphenoxyethyl))aminomethyl-1,4-benzodioxine), phentolamine (Morrow & Creese 1986; Han et al 1987a; Minneman et al 1988) and 5-methyl-urapidil (Gross et al 1988a; Hanft & Gross 1989) were designated α_{1A} (α_{1a}); those with low affinity for these compounds were designated α_{1B} (α_{1b}). The proportions of these binding sites differ in various tissues of the rat (Han et al 1987b; Gross et al 1988a) and guinea-pig (own unpublished observation). Since results on α_1-adrenoceptor subtypes postulated in functional studies and those found in radioligand binding experiments cannot yet be easily reconciled it appears desirable to investigate a variety of selective and non-selective agonists and antagonists. In the present study the binding of several compounds to α_1-adrenoceptors was characterized.

Materials and methods

[^{3}H]prazosin binding. The affinity of various agonists and antagonists for α_1-adrenoceptors of four different tissues of male Wistar rats (250–400 g) was determined by radioligand binding techniques as described previously (Gross et al 1988a, b). Freshly prepared membranes of liver, heart, cerebral cortex and hippocampus were homogenized in ice-cold buffer (50 mM Tris HCl, 2 mM EDTA, 100 mM NaCl, pH 7·4) and centrifuged at 80 000 *g* for 20 min. The pellets were resuspended by homogenization in 50 mM Tris HCl buffer containing 1 mM EDTA (pH 7·4), incubated for 10 min at 37°C and washed once more in ice-cold buffer. Crude membrane preparations were incubated in duplicate with 0·2 mM [^{3}H]prazosin and up to 19 concentrations of the competing drugs in a final volume of 1 mL at 30°C. The incubation was terminated after 45 min by rapid filtration through Whatman GF/C filters using a Brandel M 24 R cell harvester. The filters were washed twice with 15 mL of ice-cold 50 mM Tris HCl buffer (pH 7·4). The radioactivity retained on the filters was determined by liquid scintillation counting. Phentolamine (10 μM) was used to define non-specific binding which amounted to 10–20%. Preincubation in the presence or in the absence of 10 μM chloroethylclonidine (CEC) was carried out after the first homogenization for 30 min at 37°C.

Data analysis. Ligand binding data were analysed by computerized curve-fitting as described by Barlow (1983). F-Test analysis was used to decide whether a one or a two site model was more appropriate ($P<0.05$). IC50 values were transformed into K_I values according to Cheng & Prusoff (1973).

Chemicals. [^{3}H]Prazosin (specific activity 82 Ci mmol^{-1}) was purchased from NEN, Boston, MA, USA. Adrenaline bitartrate, (±)-verapamil and oxymetazoline were purchased from Sigma, Munich, FRG, chloroethylclonidine from Research Biochemicals, Natick, MA, USA, rauwolscine hydrochloride from Roth, Karlsruhe, FRG. The following drugs were kindly donated by the manufacturers: (±)amidephrine mesylate (Laevosan, Wien, Austria), butanserine (Janssen, Beerse, Belgium), I-BE 2254 (2-(3-(4-hydroxy-3-iodophenyl)ethylaminomethyl)-tetralone hydrochloride, Beiersdorf, Hamburg, FRG) phentolamine hydrochloride (Ciba-Geigy, Basel, Switzerland), prazosin hydrochloride (Pfizer, Karlsruhe, FRG), (±)YM-12617 (5-2-[[2-(o-ethoxyphenoxy)ethyl]-amino-propyl]-2-methoxybenzene-sulfonamide hydrochloride) (Yamanouchi Pharmaceutical Co., Tokyo, Japan).

Results and discussion

[^{3}H] Prazosin and [^{125}I]I-BE 2254 are extensively used as radioligands for α_1-adrenoceptors. Recently, evidence has been accumulated that α_1-adrenoceptor recognition sites labelled by these compounds do not represent a homogeneous population. Antagonists like 5-methyl-urapidil, WB-4101 and phentolamine have been shown to discriminate between two distinct binding sites (Morrow & Creese 1986; Minneman et al 1988; Gross et al 1988a, Hanft & Gross 1989). In the present study we investigated the ability of a variety of drugs to distinguish sites with high and low affinity for the aforementioned drugs which were designated α_{1A} and α_{1B}, respectively (Morrow & Creese 1986).

As shown in Table 1, butanserine, I-BE 2254, prazosin, rauwolscine and verapamil bound to a single recognition site. [^{3}H]prazosin binding was inhibited monophasically with pseudo Hill coefficients (n_H) close to unity and with similar K_I values in the four tissues investigated. In contrast, amidephrine, oxymetazoline and the potent α_1-adrenoceptor antagonist YM-12617 (Honda et al 1985, 1987) inhibited [^{3}H]prazosin binding only to liver membranes in a monophasic way but gave shallow inhibition curves with pseudo Hill coefficients significantly lower than unity (95% confidence interval) in heart, cerebral cortex and hippocampus. Two-site analysis of the data suggests the

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Table 1. Inhibition of [^{3}H]prazosin binding to membranes of different tissues of the rat by various drugs.

Tissue	Drugs	n_H	pK_{Ihigh}	pK_{Ilow}	$\%_{high}$
Liver	Prazosin	0·93±0·03		10·64±0·01	
	I-BE 2254	0·98±0·02		9·76±0·20	
	Rauwolscine	1·12±0·01		6·57±0·02	
	Butanserine	0·97±0·03		9·82±0·03	
	Verapamil	1·18±0·05		6·49±0·02	
	Amidephrine	0·96±0·02		4·75±0·03	
	Oxymetazoline	1·18±0·07		6·71±0·08	
	YM-12617	0·93±0·03		9·15±0·04	
Heart	Prazosin	1·00±0·04		10·19±0·05	
	I-BE 2254	0·99±0·03		10·06±0·03	
	Rauwolscine	1·06±0·02		6·30±0·02	
	Butanserine	0·93±0·02		9·71±0·03	
	Verapamil	1·02±0·01		6·34±0·01	
	Amidephrine	0·77±0·04	6·50±0·20	4·48±0·05	14±4
	Oxymetazoline	0·80±0·02	8·95±0·04	6·68±0·02	16±1
	YM-12617	0·79±0·02	10·35±0·21	8·98±0·02	26±1
Cerebral cortex	Prazosin	0·91±0·03		10·03±0·01	
	I-BE 2254	0·95±0·03		9·91±0·02	
	Rauwolscine	1·04±0·01		6·23±0·01	
	Butanserine	0·92±0·01		9·63±0·09	
	Verapamil	1·03±0·01		6·29±0·04	
	Amidephrine	0·65±0·02	6·14±0·19	4·45±0·12	35±3
	Oxymetazoline	0·57±0·01	8·17±0·23	6·79±0·29	40±1
	YM-12617	0·59±0·01	10·41±0·02	8·74±0·03	55±1
Hippo-campus	Prazosin	0·91±0·02		9·99±0·06	
	I-BE 2254	0·86±0·03		9·79±0·04	
	Rauwolscine	1·04±0·04		6·01±0·02	
	Butanserine	0·93±0·06		9·61±0·02	
	Verapamil	0·96±0·02		6·20±0·01	
	Amidephrine	0·62±0·03	5·96±0·06	4·37±0·08	52±5
	Oxymetazoline	0·51±0·02	8·35±0·05	6·30±0·05	61±1
	YM-12617	0·67±0·05	10·28±0·03	8·03±0·25	86+3

Membranes were incubated with 0·2 nM [^{3}H]prazosin and up to 19 concentrations of the competing ligands. $-\text{Log } K_I$ values for the high (pK_{Ihigh}) and low (pK_{Ilow}) affinity binding sites were calculated from the IC50 values according to Cheng & Prusoff (1973). In saturation experiments K_D values of [^{3}H]prazosin binding amounted to 0·02 nM in liver, 0·03 nM in heart, 0·04 nM in cerebral cortex and 0·07 nM in hippocampus ($n \geq 3$). The proportion of binding sites with high affinity is designated as $\%_{high}$. When two-site analysis of the data revealed only a single binding site for the competing ligand respective $-\log K_I$ values are referred to as pK_{Ilow}. The values represent means±s.e.m. of at least three experiments.

existence of two α_1-binding sites in these tissues. K_I values for the high (K_{Ihigh}) and for the low affinity sites ($K_{I\,low}$) for these drugs were in good agreement in the different tissues. Obviously, the low affinity sites correspond to the single binding sites found in liver which has been reported to possess only α_{1B}-adrenoceptor binding sites. YM-12617 is a racemate. However, the (−)enantiomer has been shown to have a more than 100-fold higher potency at α_1-adrenoceptors and at [^{3}H]prazosin binding sites (Honda et al 1987). Therefore, it seems unlikely that the (+)-enantiomer contributes to the biphasic inhibition curves. The proportion of binding sites with high affinity for amidephrine, oxymetazoline and YM-12617 amounted to 14–26% in the heart, to 35–55% in cerebral cortex and to 52–86% in hippocampus. They were in the same range as the amount of α_{1A}-sites determined by the use of 5-methyl-urapidil and WB-4101 (Gross et al 1988a; Hanft & Gross 1989).

The alkylating agent chloroethylclonidine (CEC) has been introduced by Minneman et al (1988) as a tool to irreversibly eliminate α_{1B}-adrenoceptor binding sites. Pretreatment of crude cortical membrane fractions with CEC decreased [^{3}H]prazosin binding by 61±2%. As expected, inhibition of [^{3}H]prazosin binding to the remaining sites by unlabelled prazosin was not altered (Fig. 1). On the other hand, inhibition curves of amidephrine and oxymetazoline became steeper indicating that these inhibitors now predominantly bound to sites with higher affinity presumably corresponding to α_{1A}-adrenoceptor binding sites.

Table 2. Inhibition of [^{3}H]prazosin binding by adrenaline.

Tissue	n_H	pK_{Ihigh}	pK_{Ilow}	$\%_{high}$
Liver	0·60±0·01	8·19±0·29	6·43±0·14	36±6
Heart	0·87±0·03	7·35±0·42	5·84±0·05	7±3
Cerebral cortex	0·76±0·02	7·35±0·14	5·73±0·02	22±1
Hippocampus	0·73±0·03	7·35±0·02	6·08±0·16	28±2

Membranes were incubated with 0·2 nM [^{3}H]prazosin and up to 19 concentrations of (−)-adrenaline. $-\text{Log } K_I$ values for the high (pK_{Ihigh}) and low (pK_{Ilow}) affinity binding sites were calculated from IC50 values according to Cheng & Prusoff (1973). The proportion of binding sites in the high-affinity form is designated as $\%_{high}$. The values represent mean ±s.e.m. of at least three experiments.

Full agonists like adrenaline and noradrenaline are known to bind to α_1-adrenoceptors with two affinity constants in various tissues, e.g. liver and heart (for literature see Gross et al 1988b). The existence of a high affinity state of the receptor for agonists which can be converted into a low affinity form by GTP is supposed to indicate coupling of receptors to N-proteins. Analysis of competition experiments with adrenaline yielded biphasic curves in all tissues examined (Table 2). However,

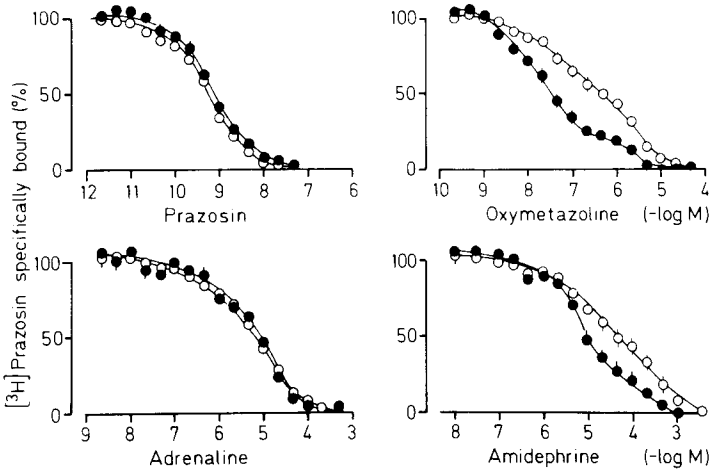

FIG. 1. [^{3}H]prazosin binding to membranes of rat cerebral cortex: effect of chloroethylclonidine (CEC) preincubation on the inhibition by unlabelled prazosin, adrenaline, amidephrine and oxymetazoline. Membranes were treated with 10 μM CEC for 30 min and subsequently washed. Given are mean $\pm$ s.e.m. of at least 4 experiments. [^{3}H]prazosin binding was decreased by 61 $\pm$ 2%, n = 16. Control (○), preincubation (●).

several observations argue that the two affinity states for adrenaline are distinct from α_{1A}/α_{1B}-binding sites: (i) The distribution of binding sites with high and low affinity in various tissues is different for adrenaline compared with amidephrine, oxymetazoline, YM-12617 (this paper), 5-methyl-urapidil and WB-4101 (Hanft & Gross 1989). (ii) In contrast to α_{1B}-binding sites, the low affinity sites for adrenaline proved to be CEC-insensitive. (iii) GTP or its analogues are known to convert high affinity sites for agonists like adrenaline into the low affinity form (own observation in liver and heart membranes) but have not been reported to affect antagonist binding. Thus, although amidephrine proved to be a partial agonist at myocardial α_1-adrenoceptors and oxymetazoline may possess some intrinsic activity at α_1-adrenoceptors of the vas deferens (Butler & Jenkinson 1978; own unpublished observation) the two binding sites calculated for these compounds (Table 1) seem to correspond to α_{1A}- and α_{1B}-binding sites rather than to the high and low affinity state of the receptor for agonists.

In conclusion, our data demonstrate that amidephrine, oxymetazoline and YM-12617 discriminate between two distinct α_1-adrenoceptor binding sites labelled by [^{3}H]prazosin (α_{1A} and α_{1B}) as already reported for 5-methyl-urapidil, WB 4101 and some other compounds. Other drugs like butanserine, I-BE 2254, prazosin, rauwolscine and verapamil display the same affinity for both binding sites. Adrenaline apparently does not discriminate between α_{1A}- and α_{1B}-binding sites. Finally, it should be pointed out that α_1-adrenoceptors with rather low affinity for prazosin have been described which may not be labelled by [^{3}H]prazosin and that α_1-adrenoceptor subtypes postulated in functional studies (for review see McGrath & Wilson 1988) may not be identical with the subtypes described by radioligand binding techniques. The compounds investigated in this study may serve as tools to clarify this issue.

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