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Heterogeneity of α_2-adrenoceptors in rat cortex but not human platelets can be defined by 8-OH-DPAT, RU 24969 and methysergide

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1 Saturation experiments indicated that [^{3}H]-yohimbine binding was specific, saturable and labelled a single population of sites in rat cerebral cortex (K_d 5.3 ± 0.9 nM, B_{max} 121 ± 10 fmol mg^{-1} protein) and human platelets (K_d 0.7 ± 0.1 nM, B_{max} 152 ± 10 fmol mg^{-1} protein).

2 The α_2-adrenoceptor antagonists, yohimbine, rauwolscine, WY 26703, idazoxan and BDF 6143 displaced [^{3}H]-yohimbine binding to each tissue in a simple manner, with high affinity and Hill slopes close to unity.

3 The α_1-adrenoceptor agonist, oxymetazoline and the antagonist prazosin inhibited the binding of [^{3}H]-yohimbine to rat cortex in a complex manner consistent with an interaction at more than one site. However, indoramin and WB 4101 only appeared to interact with one site. In contrast, in human platelets, all antagonists gave rise to monophasic displacement curves with Hill slopes close to unity suggesting a single site of interaction.

4 The 5-hydroxytryptamine (5-HT) receptor ligands, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), RU 24969, and methysergide inhibited the binding of [^{3}H]-yohimbine to rat cortex with high and low affinity, consistent with an interaction with two populations of binding sites. However, inhibition of [^{3}H]-yohimbine binding to human platelets suggested a single site of interaction. The low affinity of 5-HT, 5-carboxyamidotryptamine (5-CT) and dipropyl-5-CT indicated that [^{3}H]-yohimbine was not labelling a 5-HT$_1$-like site in rat cortex.

5 The ability of 8-OH-DPAT, RU 24969 and methysergide in addition to prazosin and oxymetazoline to differentiate [^{3}H]-yohimbine binding provides additional pharmacological evidence for heterogeneity within rat cortical α_2-adrenoceptors. However, if the two sites in rat cortex that are differentiated by the 5-HT ligands represent α_{2A}- and α_{2B}-adrenoceptor subtypes as defined by prazosin and oxymetazoline, then they do not correspond to the population of sites in human platelets. As receptor classification should be linked to affinity of drugs rather than tissue distribution, the current classification of α_2-adrenoceptor subtypes does not appear to be satisfactory.

Introduction

Amino acid sequence of the α_2-adrenoceptor (Kobilka *et al.* 1987) and molecular weight determination (Lanier *et al.*, 1988) have identified structurally distinct α_2-adrenoceptor subtypes. Cloning and gene expression studies revealed that the gene for the human platelet α_2-adrenoceptor was localised on chromosome 10 (Kobilka *et al.*, 1987) and was distinct from an α_2-adrenoceptor subtype gene localised on chromosome 4 (Regan *et al.*, 1988).

A number of radioligand binding studies have indicated that heterogeneity exists within α_2-adrenoceptors. This heterogeneity has been shown to occur between species (Feller & Bylund, 1984; Alabaster *et al.*, 1986; Dickinson *et al.*, 1986) and within species (Nahorski *et al.*, 1985; Hamilton *et al.*, 1988). The α_2-adrenoceptor antagonists [^{3}H]-yohimbine and [^{3}H]-rauwolscine bind with lower affinity to rodent tissue compared to non-rodent tissue (Bylund, 1985). Competition experiments have indicated that the affinities and rank order of a number of compounds to displace the binding of [^{3}H]-yohimbine at α_2-adrenoceptors differ between rodent and non-rodent species (Kawahara & Bylund, 1985). The α_1-adrenoceptor antagonist prazosin has been shown to be more potent as an inhibitor of [^{3}H]-yohimbine binding to α_2-adrenoceptors in certain rodent compared to non-rodent tissue, whereas oxymetazoline is more potent in non-rodent tissue (Cheung *et al.*, 1982; Bylund, 1985). Thus α_2-adrenoceptor subtypes have been differentiated on the basis of affinity for prazosin; the α_{2A} subtype has low affinity for prazosin whereas the α_{2B} has higher affinity (Cheung *et al.*, 1982; Petrash & Bylund, 1986). According to this definition, the α_2-adrenoceptor in human platelets is consistent with the α_{2A} subtype (Cheung *et al.*, 1982), whereas the α_2-adrenoceptor in neonatal rat lung tissue is of the α_{2B} subtype (Latifpour *et al.*, 1982). Rat cerebral cortex and corpus striatum appear to contain approximately equal amounts of α_{2A} and α_{2B} subtypes (Bylund, 1985).

In this study we have examined the displacement of [^{3}H]-yohimbine binding to human platelets and rat cerebral cortex by prazosin, oxymetazoline and a number of compounds known to have affinity for α-adrenoceptors and for 5-HT$_1$-like receptors.

Methods

Membrane preparation

Male Sprague-Dawley rats (180–200 g) were stunned, decapitated and the cerebral cortex dissected from the other brain regions over ice. Tissues were homogenised in 20 volumes of 50 mM Tris HCl, 5 mM EDTA, pH 7.4 with a polytron PT 10 tissue disruptor (setting 10; 2 × 10 s bursts). The homogenate was centrifuged at 48,000 *g* for 15 min at 4°C. The supernatant was discarded and the pellet resuspended in the original volume of assay buffer (50 mM Tris HCl, 0.5 mM EDTA, pH 7.4). Membranes were washed twice by repeated centrifugation at 48,000 *g* for 15 min at 4°C. The final pellet was resuspended in assay buffer at an approximate protein level of 1 mg ml^{-1}. Membranes were stored under liquid nitrogen

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Table 1 [^{3}H]-yohimbine binding to rat cortex and human platelets

	K_d (nM)	B_{max} (fmol mg^{-1} protein)
Rat cortex	5.3 ± 0.9	121 ± 10
Human platelets	0.7 ± 0.1	152 ± 10

Saturation experiments were carried out over the [^{3}H]-yohimbine concentration range 0.1–8 nM for platelets and 0.1–15 nM for rat cortex. Each value represents the meant ± s.e.mean of three determinations.

until used in the binding assay. Human platelet membranes were prepared and binding carried out as described by Cheung *et al.* (1982).

Saturation experiments

Membranes (400–600 μg protein) and [^{3}H]-yohimbine, (0.1–15 nM for rat cortex; 0.1–8 nM for human platelets) were incubated to equilibrium in 50 mM Tris HCl buffer, pH 7.4 containing 0.5 mM EDTA. Incubations were carried out for 30 min at 25°C in a final volume of 0.5 ml. Bound ligand was separated from free by vacuum filtration over GF/B filters on a Brandell cell harvester. The filters were washed twice for 10 s with incubation buffer and the bound radioactivity determined by liquid scintillation counting. Non-specific binding at each free ligand concentration was determined in the presence of 10 μM phentolamine.

Equilibrium binding parameters (K_d and B_{max}) were obtained by the iterative non-linear least square curve fitting programme 'ligand' (Munson & Rodbard, 1980).

Competition experiments

Competition experiments were carried out in the presence of 1.5 nM [^{3}H]-yohimbine and various drugs over the concentration range 1 mM to 1 pM in a final volume of 0.5 ml. Conditions for the competition assays were identical to those described for the saturation experiments. Non-specific binding was defined by 10 μM phentolamine and represented between 25–30% total binding in rat cortex and 20% in human platelets. Each determination was carried out in duplicate.

Computer analysis of displacement curves

Inhibition of specific binding of [^{3}H]-yohimbine by the drug was analysed to estimate the IC_{50} (concentration of drug displacing 50% of specific binding). The inhibitory constant (K_i) was calculated from the IC_{50} by the equation of Cheng & Prusoff (1973). Binding isotherms from displacement studies were analysed by a non-linear least square parametric curve fitting programme capable of iterative curve fitting to a single or two-site model. The programme provided a sum of squares error for a single or two site model (Michel & Whiting, 1988). The single site and two site models for each isotherm were compared by the differential F value defined by the following equation;

$$F = \frac{(SS_1 - SS_2)/(df_1 - df_2)}{SS_2/(df_2)}$$

where SS_1 is the sum of squares error for the single site, SS_2 is the sum of squares error for the two-site model, df_1 is the degrees of freedom for the single site model and df_2 the degrees of freedom for the two-site model (Munson & Rodbard, 1980; Petrash & Bylund, 1986). A two-site fit was assumed to be significantly better than a single site fit if the determined F value had a $P < 0.05$.

Protein level was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the protein standard. All data were tested for significance by Student's parametric two tailed *t* test and significance taken at $P < 0.05$.

Chemicals and drugs

[^{3}H]-yohimbine (89 Ci mmol^{-1}) was purchased from Du Pont, U.K. Drugs were obtained from the following sources; 5-hydroxytryptamine creatinine sulphate (5-HT) and oxymetazoline HCl from Sigma; rauwolscine HCl from Inverbeff; WY 26073 (N-methyl-N-(1,3,4,6,7,11b-hexahydro-2H-benzo-αaβ-quinolizin 2-yl)-s-butanesulphonamide hydrochloride) and indoramin HCl from Wyeth; idazoxan HCl from Reckitt & Colman; WB 4101 (2-[2,6-dichloro(N-beta chloroethyl-N-methyl)-4 methylamino] phenylimino-2-imidazoline dihydrochloride) from Ward Blenkinsop; 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)-tetralin hydrobromide) from Merrell Dow; RU 24969 (5-methoxy-3-(1,2,3,6, tetrahydropyridin-4-yl)-IH-indole) from Roussel; methysergide bimaleate from Sandoz; prazosin HCl from Pfizer. BDF 6143 (4-chloro-2-[2-imidazoline-2-ylamino]-isoindoline hydrochloride); 5-CT (5-

Table 2 Inhibition of [^{3}H]-yohimbine binding to human platelet and rat cortical membranes by α-adrenoceptor and 5-HT receptor ligands

	Rat cortex			*Human platelets*	
	pK_i		*nH*	pK_i	*nH*
Yohimbine	7.87 ± 0.07		0.89 ± 0.09	9.10 ± 0.07	1.03 ± 0.02
Rauwolscine	7.86 ± 0.06		0.97 ± 0.02	8.98 ± 0.03	1.08 ± 0.01
WY 26703	7.94 ± 0.07		1.02 ± 0.03	ND	
Idazoxan	7.67 ± 0.06		1.00 ± 0.05	8.30 ± 0.02	0.94 ± 0.01
BDF 6143	8.96 ± 0.04		1.03 ± 0.03	8.96 ± 0.06	1.00 ± 0.01
Prazosin	8.10 ± 0.30	5.94 ± 0.08	(50:50)	6.05 ± 0.09	0.93 ± 0.03
Oxymetazoline	8.25 ± 0.02	6.92 ± 0.13	(60:40)	8.40 ± 0.04	1.03 ± 0.04
Indoramin	5.09 ± 0.04		0.98 + 0.05)	5.90 ± 0.03	1.11 ± 0.03
WB 4101	7.24 ± 0.08		0.89 + 0.07	8.40 ± 0.04	1.01 ± 0.02
8-OH-DPAT	6.43 ± 0.07	4.97 ± 0.14	(40:60)	6.66 ± 0.07	0.97 ± 0.02
RU 24969	7.30 ± 0.11	5.20 ± 0.06	(40:60)	7.12 ± 0.10	0.97 ± 0.02
Methysergide	7.17 ± 0.09	5.34 ± 0.12	(45:55)	7.05 ± 0.11	0.95 ± 0.04
5-HT	<4.00			<4.00	
5-CT	<5.5			ND	
DP-5-CT	<5.5			ND	

The affinity values obtained for the displacement of specific binding of 1.5 nM [^{3}H]-yohimbine from rat cortical and human platelet membranes. Prazosin, oxymetazoline, 8-OH-DPAT, RU 24969 and methysergide displaced [^{3}H]-yohimbine binding to rat cortical membranes with Hill slopes (nH) that could be resolved into high and low affinity components. Values in parentheses represent the proportion of high to low affinity sites differentiated by the ligands. The pIC_{50} values for the unresolved curves were, prazosin 7.1 ± 0.10, oxymetazoline 7.7 ± 0.01, 8-OH-DPAT 5.70 ± 0.09, RU 24969 6.08 ± 0.07 and methysergide 6.16 ± 0.10. Each value represents the mean ± s.e.mean of at least four experiments. ND, not determined.

carboxyamidotryptamine maleate) and DP-5-CT (dipropyl-5-carboxyamidotryptamine hydrobromide) were synthesized by Syntex. All other chemicals were of the highest purity commercially available.

Results

Saturation analysis of [^{3}H]-yohimbine binding indicated that it was specific, saturable and bound to a single population of high affinity sites in rat cerebral cortex and human platelets. The affinity and density of α_2-adrenoceptors in rat cortex and human platelets are shown in Table 1.

The affinities of a number of drugs were determined at α_2-adrenoceptors in rat cortex and human platelets. The α_2-adrenoceptor antagonists yohimbine, rauwolscine. WY 26703, idazoxan and BDF 6143 displaced [^{3}H]-yohimbine binding to human platelets and rat cerebral cortical membranes with high affinity and with Hill slopes not significantly different from unity. The α_1-adrenoceptor antagonist indoramin and the mixed α_1/5-HT$_{1A}$ ligand WB 4101 gave rise to simple displacement curves in human platelets and rat cerebral cortical membranes, consistent with a single site of interaction. The α_1-adrenoceptor antagonist prazosin and the agonist, oxymetazoline gave rise to biphasic displacement curves indicating the possibility of two classes of binding sites. Computer analysis of the binding isotherms indicated that prazosin and oxymetazoline displaced [^{3}H]-yohimbine binding to rat cortex with high and low affinity and that these sites existed in approximately equal proportions. Differentiation of [^{3}H]-yohimbine binding by prazosin and oxymetazoline into a high and low affinity site was not apparent in human platelets. Prazosin inhibited [^{3}H]-yohimbine binding to human platelets with low affinity whereas oxymetazoline displayed high affinity (Table 2).

Inhibition of [^{3}H]-yohimbine binding to rat cortical membranes by the 5-HT agonist, 8-OH-DPAT, the mixed 5-HT$_{1A/1B}$ agonist RU 24969 and the mixed 5-HT$_1$ and 5-HT$_2$ ligand methysergide gave rise to biphasic competition curves. Computer analysis statistically resolved the binding data into a two-site fit for each of the compounds (Table 2). In contrast, displacement of [^{3}H]-yohimbine binding to human platelet α_2-adrenoceptors was monophasic, consistent with an interaction at a single site. The affinities of the 5-HT ligands at human platelet α_2-adrenoceptors corresponded to their respective values at the high affinity site on rat cortical membranes. The high affinity sites differentiated by 8-OH-DPAT, RU 24969 and methysergide are not minor components of [^{3}H]-yohimbine binding. They represent between 40 and 45% of specific [^{3}H]-yohimbine binding to rat cortical membranes. [^{3}H]-yohimbine binding to human platelet and rat cortical α_2-adrenoceptors was unaffected by 5-HT at concentrations up to 100 μM.

Methysergide, in the presence of 100 nM prazosin, no longer displaced [^{3}H]-yohimbine binding to rat cortex in a biphasic manner (Figure 1). Analysis of the displacement curves indicated low affinity binding and a Hill slope close to unity, a finding consistent with an interaction at a single site. Similarly, the high affinity component of 8-OH-DPAT and RU 24969 binding was abolished by prazosin (Table 3), suggesting

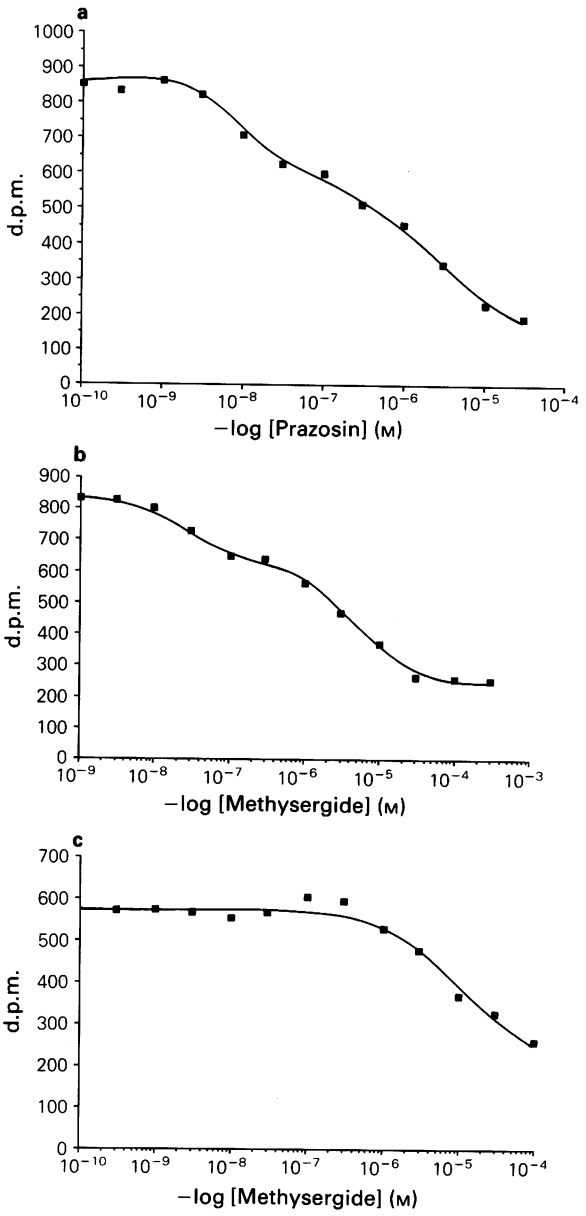

Figure 1 Displacement of [^{3}H]-yohimbine binding to rat cerebral cortical membranes by prazosin (a) and by methysergide in the absence (b) and presence (c) of 100 nM prazosin. Computer assisted curve fitting on the prazosin displacement curve (a) demonstrated that a two-site fit was significantly better ($P < 0.01$) than a one-site fit. (1 site, SS$_1$ 9483; 2 site, SS$_2$ 3684; F value 6.29). In the absence of prazosin (b), computer assisted curve fitting showed a two-site fit was significantly better ($P < 0.01$) than a one-site fit for the displacement of [^{3}H]-yohimbine by methysergide. (1 site, SS$_1$ 11212, d.f. 10; 2 site, SS$_2$ 4342, d.f. 8: F value 6.32). Prazosin decreased specific [^{3}H]-yohimbine binding by approximately 40%. Methysergide, in the presence of prazosin (c), inhibited [^{3}H]-yohimbine binding with a Hill slope of unity and showed a one-site fit. The data describe a single experiment performed in duplicate.

Table 3 [^{3}H]-yohimbine binding to rat cortical and human platelet membranes in the presence of 100 nM prazosin

	pK_i			
	Rat cortex	*nH*	*Human platelets*	*nH*
Oxymetazoline	8.07 ± 0.03	1.00 ± 0.04	8.56 ± 0.11	0.93 ± 0.07
8-OH-DPAT	5.07 ± 0.03	0.97 ± 0.04	6.34 ± 0.04	1.02 ± 0.06
RU 24969	5.38 ± 0.04	0.89 ± 0.07	6.84 ± 0.06	0.98 ± 0.03
Methysergide	5.38 ± 0.04	0.94 ± 0.05	7.09 ± 0.10	1.03 ± 0.06

Binding of 1.5 nM [^{3}H]-yohimbine to rat cortical and human platelet membranes was carried out in the presence of 100 nM prazosin. The affinity values were obtained from the displacement of the remaining specific binding to human platelet and rat cortical membranes. Specific binding of [^{3}H]-yohimbine to platelet membranes was unaffected by prazosin. Each value represents the mean ± s.e.mean of at least four experiments. nH represents the Hill slope of the displacement curves.

Table 4 Effect of GTP on the affinity values for α_2-adrenoceptor binding sites in rat cortical membranes

	pIC_{50}	
	$-GTP$	$+GTP$
Adrenaline	7.63 ± 0.05	5.95 ± 0.01*
Noradrenaline	6.81 ± 0.05	6.12 ± 0.05*
Prazosin	7.10 ± 0.10	7.05 ± 0.10
8-OH-DPAT	5.70 ± 0.09	5.70 ± 0.08
RU 24969	6.08 ± 0.07	5.90 ± 0.10
Methysergide	6.16 ± 0.10	5.90 ± 0.10

The affinity values were obtained for specific [^{3}H]-yohimbine (1.5 nM) binding to rat cortical membranes in the presence (+) and absence (−) of 0.1 mM GTP. The results are expressed as pIC_{50} since the compounds showed Hill slopes that were significantly ($P < 0.001$) different from unity. Values shown are the mean ± s.e.mean of at least three experiments. Statistical significance relative to the value in the absence of GTP, *$P < 0.001$.

that the site showing high affinity for the 5-HT$_1$ ligands and prazosin were likely to be identical. In contrast, oxymetazoline in the presence of prazosin, still retained high affinity for [^{3}H]-yohimbine binding but this time gave rise to monophasic displacement curves. Thus the site displaying high affinity towards oxymetazoline is different from the sites showing high affinity towards prazosin and the 5-HT$_1$ ligands. The inclusion of 100 nM prazosin was repeated in human platelets. Prazosin had no significant effect on the affinity of the 5-HT$_1$ ligands. The affinity of each ligand, even in the presence of prazosin, was significantly higher at the α_2-adrenoceptors on human platelets than at the prazosin-insensitive site in rat cortex.

A possible explanation for the complex displacement curves obtained with prazosin, oxymetazoline and the 5-HT$_1$ ligands is that they act as agonists at one or both of the [^{3}H]-yohimbine binding sites. Displacement studies were repeated in the presence of 0.1 mM guanosine 5'-triphosphate (GTP). The potency of adrenaline and noradrenaline to displace [^{3}H]-yohimbine binding from cortical membranes was reduced significantly in the presence of GTP (Table 4). In contrast GTP failed to alter the inhibition curves for prazosin, 8-OH-DPAT, methysergide and RU 24969.

Discussion

Prazosin has been shown previously to inhibit [^{3}H]-yohimbine binding to rat cerebral cortex in a biphasic manner, with an approximate 40 fold separation between the high (pK_i 8.2) and low (pK_i 6.6) affinity site (Bylund, 1985). The affinity of prazosin at each site and the proportion of the subtypes identified in this study, is in agreement with the findings of Bylund (1985) and supports the view that multiple α_2-adrenoceptor subtypes exist in rat cerebral cortex.

The results of this study also demonstrated that the 5-HT receptor ligands, 8-OH-DPAT, RU 24969 and methysergide, but not 5-HT, 5-CT or DP-5-CT, inhibited the binding of [^{3}H]-yohimbine to rat cortex in a complex manner. Computer analysis of the displacement curves demonstrated that each drug inhibited [^{3}H]-yohimbine binding with high and low affinity. Binding of [^{3}H]-yohimbine to the high affinity site differentiated by these ligands was inhibited by 100 nM prazosin. Under these conditions 8-OH-DPAT, RU 24969 and methysergide no longer displaced [^{3}H]-yohimbine binding to rat cortical membranes with high and low affinity. The resulting monophasic displacement curves were of low affinity. Oxymetazoline, in the presence of 100 nM prazosin, also displayed monophasic displacement curves, but unlike the 5-HT$_1$ ligands retained high affinity towards the binding site labelled by [^{3}H]-yohimbine. Based on the α_2-adrenoceptor subtype classification proposed by Bylund (1985) the [^{3}H]-yohimbine binding site that displayed high affinity towards the 5-HT$_1$ ligands would correspond to the α_{2B}-adrenoceptor subtype (high affinity for prazosin), whereas the site that displayed low affinity towards the 5-HT$_1$ ligands would equate with the α_{2A}-adrenoceptor subtype (low affinity for prazosin). Occlusion experiments were repeated with oxymetazoline instead of prazosin, to determine if the 5-HT$_1$ ligands retained only high affinity towards the [^{3}H]-yohimbine binding site in rat cerebral cortex. Oxymetazoline was found to be unsuitable in this type of experiment, as the difference in affinity of oxymetazoline at the prazosin sensitive and insensitive sites was not sufficient to allow a concentration to be chosen which would selectively block one site. Occlusion experiments to prevent [^{3}H]-yohimbine binding to α_{2A}-adrenoceptors must await the identification of more selective ligands than oxymetazoline. The ability of 8-OH-DPAT, RU 24969 and methysergide to differentiate [^{3}H]-yohimbine binding provides additional pharmacological evidence that [^{3}H]-yohimbine labels with high affinity more than one site in rat cortex.

Human platelet α_2-adrenoceptors have been labelled with [^{3}H]-yohimbine (Motulsky *et al.*, 1980; Daiguji *et al.*, 1981). Saturation experiments indicate the presence of a single population of high affinity α_2-adrenoceptors (Boon *et al.*, 1983; Kerry *et al.*, 1984). The low affinity displayed by prazosin towards the [^{3}H]-yohimbine binding site in human platelets is the basis of the suggestion that platelets contain the α_{2A}-adrenoceptor subtype (Cheung *et al.*, 1982). In the present study a paradox is evident. First, the low affinity displayed by prazosin indicated that the platelet site can be designated as α_{2A}, as demonstrated by others (Cheung *et al.*, 1982), whereas the affinities of the 5-HT$_1$ ligands at the α_2-adrenoceptor in human platelets were closer to the site previously considered to be the α_{2B}-adrenoceptor subtype in rat cortex. The inclusion of 100 nM prazosin had no effect on the specific [^{3}H]-yohimbine binding to human platelets or on the affinity of the 5-HT$_1$ ligands for this site, thus confirming the α_{2A}-adrenoceptor nature of this site. The failure to define clearly the nature of the sites may relate to species differences with the existence of several sub-classes of sites. The human platelet α_2-adrenoceptor may itself be somewhat atypical. The dissociation constant for [^{3}H]-yohimbine binding was considerably lower in human platelets than in rat cortex. At present the affinities of the 5-HT$_1$ ligands are not entirely consistent with a simple α_{2A}- and α_{2B}-adrenoceptor classification.

Evidence for [^{3}H]-yohimbine binding to more than one site was apparent only from displacement studies. Saturation experiments, over the [^{3}H]-yohimbine concentration range 0.1 to 15 nM, failed to demonstrate binding to more than one site in rat cortex. Analysis of the binding isotherms indicated that [^{3}H]-yohimbine labelled a uniform population of binding sites. [^{3}H]-yohimbine has been shown to label a single population of binding sites in rat (Cheung *et al.*, 1982; Rouot *et al.*, 1982) and human cortex (Petrash & Bylund, 1986). The failure to identify more than one population of binding sites in saturation studies implies either that [^{3}H]-yohimbine binds with equal affinity to more than one site or that the difference in affinity between sites is not sufficient to be resolved by the analysis programmes used. In human caudate nucleus, a tissue suggested to contain two α_2-adrenoceptor subtypes, saturation experiments, although giving rise to curvilinear Rosenthal plots, failed to demonstrate that the binding of [^{3}H]-yohimbine was significantly better as a two-site than a one-site fit (Petrash & Bylund, 1986).

Saturation studies carried out over a higher [^{3}H]-yohimbine concentration range (0.2 to 80 nM) than that used in this study (0.1–15 nM) have demonstrated binding to a second site in rat cortex (Michel & Whiting, 1984). In addition to [^{3}H]-yohimbine, [^{3}H]-rauwolscine at high concentrations has been shown to bind in a biphasic manner to rat cortical membranes (Diop *et al.*, 1983). Binding isotherms generated over the [^{3}H]-rauwolscine concentration range 0.25–50 nM were consistent with binding to a high and low affinity site (Broadhurst & Wyllie, 1986). The finding that [^{3}H]-rauwolscine binding to the low affinity site was inhibited by 300 nM spiroperidol led

Broadhurst & Wyllie (1986) to conclude that [^{3}H]-rauwolscine, in addition to binding to α_2-adrenoceptors, also labelled 5-HT_2 receptors. It is unlikely that either of the [^{3}H]-yohimbine binding sites differentiated by prazosin or the 5-HT_1 ligands are 5-HT_2 receptors. Inclusion of 300 nM spiroperidol did not alter the affinity of prazosin or the 5-HT ligands, 8-OH-DPAT, RU 24969 and methysergide for the high or low affinity sites in rat cortex (data not shown). Recent studies have shown that 5-HT can displace [^{3}H]-rauwolscine binding from a non α_2-adrenoceptor site in human cortex with nanomolar affinity (Convents *et al.*, 1988; 1989) and that [^{3}H]-rauwolscine may label 5-HT_{1A} receptors in rat cerebral cortex (Broadhurst *et al.*, 1988). Yohimbine has also been shown to possess high affinity (59 nM) towards the 5-HT_{1D} binding site identified in non-rodent brain (Heuring & Peroutka, 1987). The low affinity of 5-CT and DP-5-CT and the failure of 5-HT, at concentrations up to 100 μM, to displace [^{3}H]-yohimbine binding to rat cortical membranes argues against either of the sites being 5-HT_1-like in nature. The displacement of [^{3}H]-yohimbine binding to rat cortical membranes by 5-HT was also studied under conditions in which 5-HT_1-like sites are labelled by [^{3}H]-5-HT (50 mM Tris HCl buffer, pH 7.4 containing 5.7 mM ascorbic acid, 4 mM $CaCl_2$ and 10 μM pargyline). The affinity of 5-HT was not increased under these conditions (data not shown), indicating that the low affinity of 5-HT is the result of a failure to displace [^{3}H]-yohimbine and not from either metabolism or oxidation of 5-HT. The ability of WY 26703, idazoxan and BDF 6143, in addition to rauwolscine and yohimbine, to displace [^{3}H]-yohimbine binding to cortical membranes with high affinity, is consistent with the radioligand labelling an α_2-adrenoceptor. The α_2-adrenoceptor subtype with 'high' affinity for prazosin is not related to the α_1-adrenoceptor, as this receptor displays a further 250 times higher affinity for prazosin (Petrash & Bylund, 1986).

Evidence indicates that α_2-adrenoceptor agonists form a ternary complex with the receptor and inhibitory guanine-nucleotide protein (Gi) resulting in a high affinity state of the receptor (Hoffman *et al.*, 1982; Bylund & U'Prichard. 1983). Guanine nucleotides act by inhibiting the formation/or destabilizing the receptor-guanine nucleotide protein complex to a low affinity receptor state. In this study, the potency of adrenaline and noradrenaline to displace [^{3}H]-yohimbine from its binding site was reduced significantly in the presence of 0.1 mM GTP. In contrast the displacement curves for 8-OH-DPAT, RU 24969 and methysergide were unaffected by GTP, suggesting that different affinity states or agonist interactions are unlikely to account for the biphasic displacement curves obtained with the 5-HT ligands. It has been shown previously that the biphasic displacement of [^{3}H]-yohimbine binding to rat brain by prazosin does not involve negative cooperativity or different affinity states of the α_2-adrenoceptor (Bylund, 1985). It remains to be determined if 8-OH-DPAT, RU 24969 and methysergide act at an allosteric binding site influencing α_2-adrenoceptor binding rather than competing directly at the site itself.

The results of this study provide additional evidence that human platelets contain a single population of α_2-adrenoceptors whereas rat cerebral cortex contains a mixed population of α_2-adrenoceptor subtypes. The ability of 8-OH-DPAT, RU 24969 and methysergide, in addition to prazosin and oxymetazoline, to differentiate [^{3}H]-yohimbine binding provides additional pharmacological evidence for heterogeneity within rat brain α_2-adrenoceptor binding sites and thus possibly within functional α_2-adrenoceptors. Sequencing studies have indicated that the 5-HT_{1A} receptor is closely related to the structural core for adrenoceptors (Fargin *et al.*, 1988) and it is therefore to be expected that there will be some overlap in binding affinities for the 5-HT_{1A} receptor and subtypes of α_2-adrenoceptor. If the two sites defined in rat cortex by the 5-HT ligands represent the α_{2A}- and α_{2B}-adrenoceptor subtypes, then they do not correspond to the population of sites that exists on human platelets. Alternatively, if the human platelet adrenoceptor is considered to be the α_{2A}-adrenoceptor subtype, then the subtypes defined in rat cortex, by the 5-HT ligands, do not fit into the current classification for α_2-adrenoceptor subtypes.

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