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Binding of yohimbine stereoisomers to α-adrenoceptors in rat liver and human platelets

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1 Displacement of tritiated prazosin binding to rat liver plasma membranes and tritiated yohimbine human platelet membranes shows that (+)-yohimbine, alloyohimbine and α-yohimbine (rauwolscine) are selective α_2-adrenoceptor antagonists ($K_D\alpha_1/K_D\alpha_2$: 635, 46.6 and 112 respectively) whereas corynanthine is more α_1-selective ($K_D\alpha_1/K_D\alpha_2$: 0.036).

2 11-Methoxy derivatives of α-yohimbine and epi-α-yohimbine are very weak α-adrenoceptor blockers.

3 It is concluded that the aromatic A ring, the Nb atom, and the carboxymethyl moiety are important for the binding of yohimbine to the α-adrenoceptor, the carboxymethyl group being important for the α_1/α_2 specificity of the molecule.

Introduction

Yohimbine has been shown to be a highly selective α_2-adrenoceptor antagonist (Starke, Borowski & Endo, 1975) and tritiated yohimbine has been extensively used to characterize the α_2-receptor in various tissues (Motulsky, Shattil & Insel, 1980; Mukherjee, 1980; Daiguji, Meltzer & U'Prichard, 1981; Hoffman, Dukes & Lefkowitz, 1981). However, yohimbine contains five asymmetrical carbons and therefore exists in a number of isomeric forms (Figure 1). It has been demonstrated that the various isomers possess different physiological properties (Lambert, Lang, Friedman, Meller & Gershon, 1978) and, in particular, act differently on the α_1- and α_2- subtypes of the α-adrenoceptor (Weitzell, Tanaka & Starke, 1979; Shepperson, Duval, Massingham & Langer, 1981). Although yohimbine and two of its isomers, corynanthine and α-yohimbine (or rauwolscine), have been widely used to characterize the α_1/α_2 specificity of various ligands and physiological responses (Tanaka & Starke, 1980; Hedler, Stamm, Weitzell & Starke, 1981; Timmermans, Schoop, Kwa & Van Zwieten, 1981), the affinity of the isomers for the α_1- and α_2-adrenoceptors has not been determined by *in vitro* binding studies.

In an attempt to gain a better understanding of the structure-activity relationship of the yohimbine stereoisomers, we have determined the affinity of each isomer for the α_1- and α_2-adrenoceptors using optimal binding conditions. Firstly, binding affinity of the yohimbine isomers to the α_1- and α_2-receptors was measured by displacement of the highly selective α_1-antagonist, prazosin (Brogden, Heel, Speight & Avery, 1977) and α_2-antagonist, yohimbine, respectively. Moreover membrane preparations were used, rat liver plasma membranes and human platelet membranes, which contain almost exclusively a single class of α-adrenoceptor. Rat liver plasma membranes possess α-adrenoceptors which are predominantly (80%) of the α_1-subtype (Hoffman, Mullikin-Kilpatrick & Lefkowitz, 1980). In contrast, human platelet membranes appear to contain only α_2-adrenoceptors (Hoffman, DeLean, Wood, Schocken & Lefkowitz, 1979). Using these two membranes systems, we have been able to determine the affinity of the yohimbine stereoisomers for α_1- and α_2-adrenoceptors, and to obtain greater insight into the structure-activity relationship of yohimbine isomers.

Methods

Preparation of rat liver plasma membranes

Rat liver plasma membranes were prepared from female, albino, Wistar rats (100–150 g body weight) according to the procedure devised by Neville (1968) up to step 11. The membrane preparations were stored in liquid nitrogen until use.

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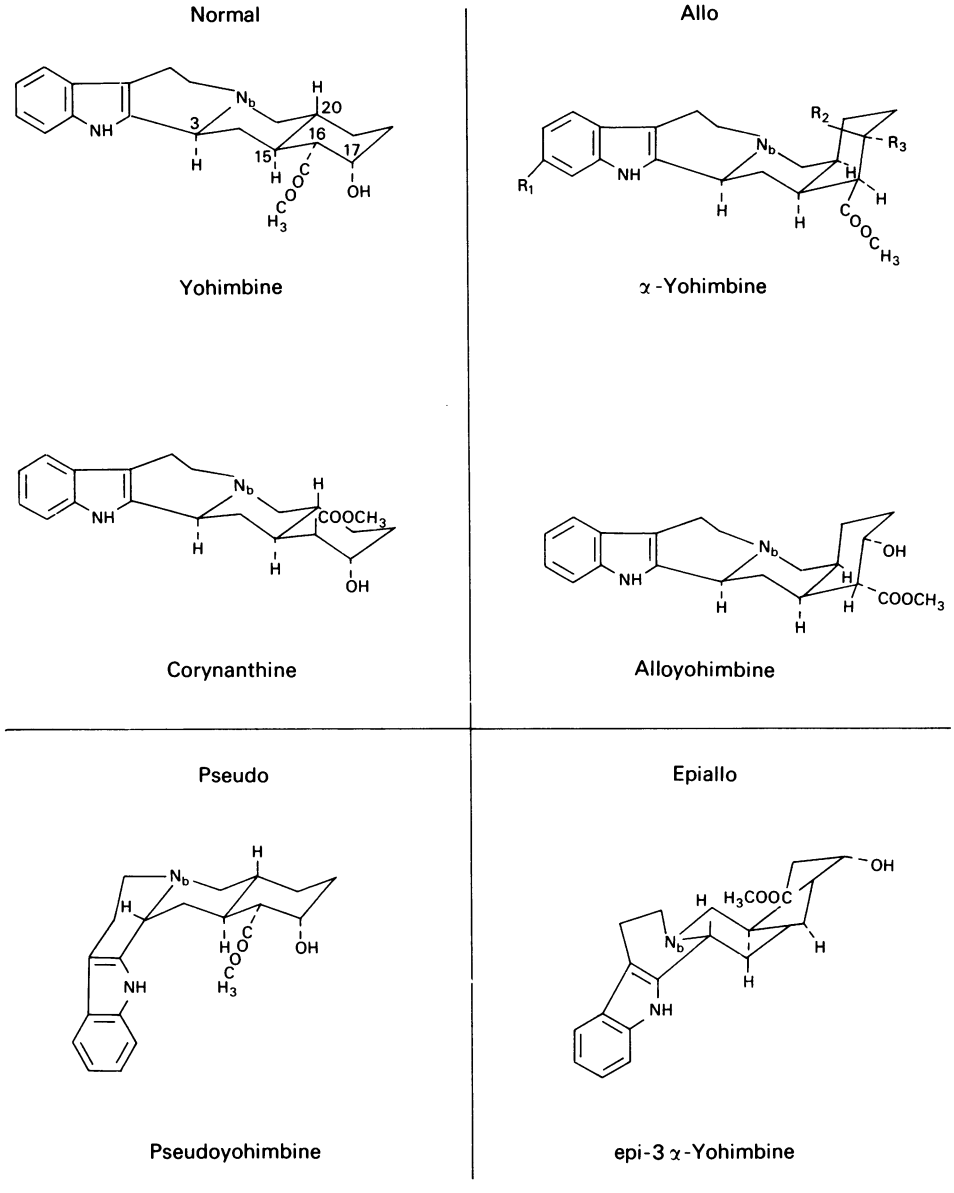

Figure 1 Chemical structures of the yohimbine stereoisomers. Normal series corresponds to the 3α, 15α, 20β configuration; allo series to the 3α, 15α, 20α; pseudo series to 3β, 15α, 20β; and epiallo series to 3α, 15α, 20α. In the allo series: $R_1 = R_2 = H$, $R_3 = OH$: α-yohimbine (rauwolscine); $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$: 11-methoxy-α-yohimbine; $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$: 11-methoxy-17-epi-α-yohimbine.

Preparation of human platelet membranes

Human platelet membranes were prepared from young, healthy, male donors (24–35 years old). Sixty ml of venous blood was collected in a plastic syringe containing 2 ml of 100 mM disodium edetate (EDTA). Platelet-rich plasma was prepared by differential centrifugation for 10 min at 500 *g*, at room temperature. Platelet-rich plasma was then removed and mixed with an equal volume of washing buffer containing 135 mM NaCl, 13 mM sodium citrate, 5 mM glucose and 1 mM EDTA, adjusted to pH 7.5. Platelets were then pelleted by centrifugation for 10 min at 600 *g* and resuspended in a hypotonic

buffer containing 5 mM Tris-HCl, pH 7.4 and 5 mM EGTA. The resulting platelet homogenate was then rapidly frozen in liquid nitrogen and thawed at 20°C (operation repeated three times). Lysed platelets were then washed by successive contrifugation steps (30,000 *g*, 20 min) in the same medium. The final pellet was resuspended in the incubation buffer. Membrane protein was determined according to Lowry's procedure, using bovine serum albumin as standard.

Binding assays

The binding of tritiated prazosin to liver plasma membranes was carried out as previously described (Geynet, Ferry, Borsodi & Hanoune, 1981) with the following modifications: liver membranes (50–100 μg) were incubated for 10 min at 25°C in a final volume of 400 μl containing 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, in the presence of tritiated prazosin (1 nM) and varying concentrations of drugs. At the end of the incubation period, each assay was diluted with 10 ml of buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4) and immediately filtered under vacuum through Whatman GF/C glass fibre filters. Filters were rapidly washed with 10 ml of the same buffer and counted in 10 ml of ready-Solv EP (Beckman) liquid scintillation mixture.

The binding of tritiated yohimbine to human platelet membranes was carried out as previously described (Insel, Stengel, Ferry & Hanoune, 1982).

For both ligands, specific binding was defined as the difference between binding of the radioligand in the absence and in the presence of 10 μM phentolamine. All values in the text refer to specific binding.

In competition studies, K_D values for the drugs were calculated according to Cheng & Prusoff (1973).

Drugs

Alloyohimbine was a gift from Prof. J. Poisson (Faculté de Pharmacie, Chatenay-Malabry, France); Pseudoyohimbine and 3-epi alloyohimbine extracted from *Alstonia quaternata* (Heurck and Muell-Arg), a plant of the Apocynaceae collected in Vanuatu (Mamatas-Kalamaras, Sevenet, Thal & Potier, 1975) were kindly donated by the Institut des Chimie des Substances Naturelles (CNRS, Gif sur Yvette, France); 11-methoxy 17-epi-α-yohimbine and 11-methoxy-α-yohimbine, extracted from *Neisosperma glomerata* (Blume, Fosberg & Sachet) (Apocynaceae) collected in Indonesia (Bogar) (Seguin, Koch & Sevenet, 1982), were obtained as gifts from Prof. M. Koch (Faculté de Pharmacie, Paris, France). All the above alkaloids were used as tartaric acid salts. Phentolamine (Ciba-Geigy), α-yohimbine hydrochloride (Chem. Service), (+)-yohimbine (Boehringer) were obtained as gifts; corynanthine hydrochloride, EGTA (ethylene glycol bis (beta-aminoethyl ether) NNN'N' tetraacetic acid) were purchased from Sigma.

Tritiated prazosin (28 Ci/mmol) was supplied by the Radiochemical Centre (Amersham) and tritiated yohimbine (82.6 Ci/mmol) by New England Nuclear Co (Boston, MA). All other chemicals were from Merck (Darmstadt, West Germany) and of analytical grade.

Results

We have previously shown that, at the concentration of tritiated prazosin used in this study (1 nM), binding occurred only to a single class of hepatic α_1-adrenoceptors with a maximal number of sites (*Bmax*) of 700 fmol/mg protein and a dissociation constant (K_D) of 0.1 nM (Geynet *et al.*, 1981). Figure 2 shows the displacement of tritiated prazosin binding by corynanthine, α-yohimbine, (+)-yohimbine and alloyohimbine. The affinities and order of potency obtained: corynanthine (K_D = 20 nM) ⩾ (+)-yohimbine (K_D = 127 nM) > alloyohimbine (K_D = 280 nM) > α-yohimbine (K_D = 336 nM), clearly demonstrate that corynanthine, but not the other yohimbine isomers, behaves as a potent α_1-adrenoceptor antagonist.

The binding of tritiated yohimbine to human

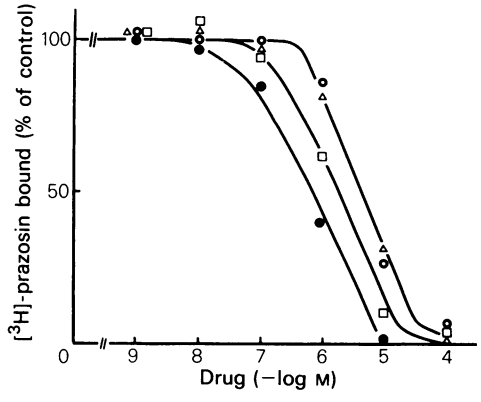

Figure 2. Competition of yohimbine isomers for tritiated prazosin binding sites in rat liver membranes. Liver membranes (60–85 μg protein) were incubated as described in Methods with tritiated prazosin (1 nM) in the presence of various concentrations of drugs. Values, which are mean of triplicate determinations, are expressed as percentage of control binding in the absence of drug (100% = 700 ± 118 fmol/mg protein): (●) corynanthine; (□) (+)-yohimbine; (△) alloyohimbine; (○) α-yohimbine.

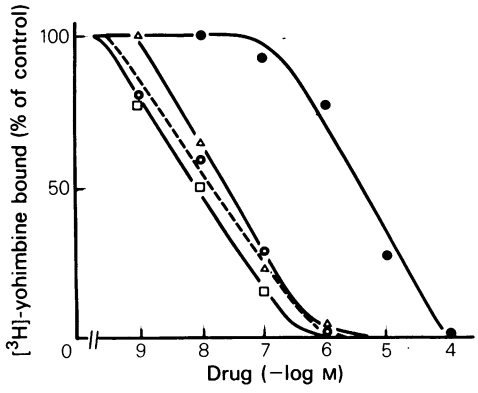

Figure 3 Competition of yohimbine isomers for tritiated yohimbine binding sites in human platelet membranes. Human platelet membranes (25–50 μg protein) were incubated as described in Methods with tritiated yohimbine (7 nM) in the presence of various concentrations of drugs. Values, which are mean of triplicate determinations are expressed as percentage of control binding in the absence of drug (100% = 230 fmol/mg protein). Symbols are as in Figure 2 (dotted lines are for ○).

platelet membranes has a maximal number of sites (*Bmax*) of 210 ± 50 fmol/mg protein with a K_D value of 2.7 ± 0.7 nM (Motulsky *et al.*, 1980). In Figure 3 are depicted the displacement curves obtained with yohimbine isomers. The order of potency demonstrates that (+)-yohimbine, α-yohimbine and alloyohimbine are equipotent α_2-adrenoceptor antagonists, with K_D values of 2 nM, 3 nM and 6 nM respectively, where as corynanthine appears to be much less effective (K_D = 557 nM).

The affinities of the yohimbine isomers and derivatives for the α_1-and α_2-adrenoceptors are summarized in Table 1. The results show that α-yohimbine, (+)-yohimbine and alloyohimbine are selective α_2-adrenoceptor antagonists whereas corynanthine appears to have a greater α_1-specificity. Pseudoyohimbine and epi-alloyohimbine are very weak adrenoceptor antagonists both at α_1- and α_2-sites. The 11 methoxy derivatives of both α-yohimbine and 17-epi-α-yohimbine are weak α_2-antagonists and have almost no affinity for α_1-adrenoceptors.

Discussion

The present work constitutes a biochemical assessment of the potencies of yohimbine isomers at the α_1 -and α_2-adrenoceptors. To date, various studies have been performed using yohimbine isomers to characterize the α_1/α_2 selectivity of adrenergic physiological responses (Hedler *et al.*, 1981). Timmermans *et al.* (1981) and Tanaka & Starke (1980) have proposed the use of yohimbine isomers to determine the α_1- or α_2-selectivity of radiolabelled ligand. The latter studies were based on the assumption that, of yohimbine isomers, corynanthine, but not yohimbine and α-yohimbine (rauwolscine), behaves as a potent α_1-antagonist, whereas yohimbine and α-yohimbine are α_2-selective antagonists (Weitzell *et al.*, 1979; Shepperson *et al.*, 1981). These results have been obtained by a pharmacological approach using preparations which contain both α_1- and α_2-receptors. From our results, α-yohimbine, (+)-yohimbine and alloyohimbine are selective α_2-antagonists with, respectively, 112, 63 and 47 fold greater affinity at α_2- than α_1-binding sites. In contrast, corynanthine appears to be more α_1-selective, but its affinity for the α_1-adrenoceptor site is relatively low, having a K_D of 20 nM. Furthermore, the affinity of corynanthine for α_1-adrenoceptors is only 28 fold greater than for α_2-receptors (Table 1) and 6 fold greater than that of (+)-yohimbine for α_1-adrenoceptors (Figure 2). This drug appears to be rather a poor tool for characterizing this type of receptor as compared to prazosin (K_D = 0.1 nM for α_1-adrenoceptor (Geynet *et al.*,

Table 1 K_D values of yohimbine stereoisomers for α_1- and α_2-adrenoceptors

Drug	K_D α_1 (μM)	K_D α_2 (μM)	K_D $\alpha_1/K_D\alpha_2$
α-Yohimbine	0.336	0.003	112
(+)-Yohimbine	0.127	0.002	63.5
Alloyohimbine	0.280	0.006	46.6
Corynanthine	0.020	0.557	0.036
Pseudoyohimbine	0.820	0.928	0.88
Epialloyohimbine	1.6	1.45	1.10
11-Methoxy-17-epi-α-yohimbine	11.81	0.375	31.5
11-Methoxy-α-yohimbine	5.29	0.160	33

K_D values of yohimbine stereoisomers and derivatives for α_1-and α_2-adrenoceptors. Liver membranes (α_1) or human platelets membranes (α_2) were incubated as described in Methods in the presence of varying concentrations of each drug. K_D values were calculated by the method of Cheng & Prusoff (1973).

1981)), which is highly selective for α_1-adrenoceptors ($K_D = 2000$ nM for α_2-receptor (Motulsky *et al.*, 1980)).

Our results also demonstrate that only stereoisomers of the 'normal' and 'allo' series are potent α-adrenoceptor blockers, whereas the 'pseudo' and 'epiallo' derivatives are weak antagonists of both types of α-adrenoceptors. If we examine, using Dreiding models, the spatial structure of these alkaloids (Figure 1), and consider, as suggested by Easson & Stedman (1933) and McGrath (1982), that the indole nucleus, nitrogen atom Nb and carboxymethyl substituent at 16-C are important for binding to the adrenoceptor, we can make the following observations: (1) yohimbine (from 'normal' series), α-yohimbine and alloyohimbine (from 'allo' series) have these three sites, i.e. indole nucleus, Nb atom, and the carbonyl 0 of 16-carboxymethyl substituent, in the same medium plane. In contrast corynanthine ('normal' series) differs from yohimbine in the beta axial position of the 16-carbomethoxy substituent, which means that no conformational change can bring the carbonyl-0 of 16-carbomethoxy substituent in the same plane as the two other postulated binding moieties. Therefore, in the 'normal' series, the α_1/α_2 specificity of yohimbine isomers may be determined by the 16-carbomethoxy configuration. (2) The 'pseudo' and 'epiallo' alkaloids do not have a planar structure, and, therefore, do not bind to the receptor with high affinity. (3) Methoxy substitution of the A ring yields compounds that are inactive on both subtypes of α-adrenoceptors (Table 1). This suggests that the electrondonor effect of the methoxy group leads to a delocalization of the π system of the indole nucleus. Therefore in these compounds the possible role of the indole nucleus in binding to a corresponding aromatic moiety of the receptor site is impaired. It appears that only slight modifications of the yohimbine molecule can affect its selectivity, suggesting that α_1- and α_2-adrenoceptors are closely related entities. Perhaps these two receptors are derived from a common ancestral protein, genetically modified during evolution?

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