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α_2-ADRENORECEPTORS ON HUMAN PLATELETS: SELECTIVE LABELLING BY [^{3}H]CLONIDINE AND [^{3}H]YOHIMBINE AND COMPETITIVE INHIBITION BY ANTIDEPRESSANT DRUGS *

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[^{3}H]Clonidine and [^{3}H]yohimbine have been used to characterize α_2-adrenoreceptors on human platelets. At 25°C binding was rapid ($t_{1/2}$ of association, 1.8 and 2.7 min) and reversible ($t_{1/2}$ of dissociation, 0.5 and 8.2 min). The binding sites for [^{3}H]clonidine and [^{3}H]yohimbine showed the specificity required for an α_2-adrenoreceptor. The rank order of potency of inhibitors of [^{3}H]clonidine binding was clonidine > yohimbine ≫ phenylephrine > prazosin and of [^{3}H]yohimbine binding was yohimbine > clonidine ≫ phenylephrine > prazosin. Scatchard analysis of [^{3}H]yohimbine binding indicated the existence of a single population of noninteracting sites ($K_D = 3.0$ nM; $B_{max} = 188$ fmol/mg protein). The high-affinity binding of [^{3}H]clonidine had a lower affinity and a lower number of sites ($K_D = 5.0$ nM; $B_{max} = 35$ fmol/mg protein). [^{3}H]Clonidine binding also showed evidence of a second site of much lower affinity and greater number ($K_D = 18.6$ nM; $B_{max} = 77$ fmol/mg protein) in 40% of the normal population. In vitro, antidepressant drugs competed with [^{3}H]clonidine and [^{3}H]yohimbine for the platelet α_2-adrenoreceptor. The rank order of potency of inhibitors of [^{3}H]clonidine binding was mianserin > amitriptyline ≫ iprindole > desipramine and of [^{3}H]yohimbine binding was mianserin > amitriptyline ≫ desipramine > iprindole. The inhibition constants (K_i) of adrenergic drugs and of various antidepressant drugs in competing with [^{3}H]clonidine were correlated with the inhibition constants of these drugs in competing with [^{3}H]yohimbine ($r = 0.970$; $P < 0.001$) which suggests that both radioligands labelled the same α_2-adrenoreceptor on the human platelet. The inhibition of binding induced by all antidepressant drugs was competitive. In contrast, long-term administration of tricyclic antidepressant drugs to patients was recently found to be associated with a decrease in the number of binding sites for [^{3}H]clonidine on platelet membranes. The present results indicate that both [^{3}H]clonidine and [^{3}H]yohimbine are useful tools for the quantification of α_2-adrenoreceptors on blood platelets and suggests that the specific binding of radiolabelled α_2-adrenoreceptor ligands to human platelet membranes might be used to monitor changes in α_2-adrenoreceptors during tricylic antidepressant drug treatment.

α_2-Adrenoreceptor Blood platelet [^{3}H]Clonidine [^{3}H]Yohimbine Antidepressant drugs

1. Introduction

The long-term administration of amitriptyline decreases the number of high affinity binding sites for [^{3}H]clonidine in neural membranes isolated from specific areas of the rat brain (Smith et al., 1981). This reduction in the number of α_2-adrenoreceptors appears to be the molecular mechanism by which the chronic administration of tricyclic antidepressant drugs increases the neuronal release of norepinephrine. This increased release of norepinephrine previously was shown to be secondary to the development of presynaptic α_2-adrenoreceptor subsensitivity (Crews and Smith, 1978, 1980). Since desensitization of the α_2-adrenoreceptor might be relevant to the clinical effectiveness of tricyclic drugs, the assessment of the function of this receptor in a readily available peripheral tissue is of both theoretical and practical value.

The human blood platelet has been used as a model to indirectly evaluate changes in nerve cell function in patients with psychiatric disorders

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(Stahl, 1977). Among other neuron-like characteristics, the human blood platelet possesses α-adrenoreceptors which when stimulated appear to be responsible for initiation of platelet aggregation induced by physiological catecholamines (Ardlie et al., 1966). Recently, α-adrenoreceptors on human platelets have been characterized by means of receptor binding techniques with [^{3}H]dihydroergocryptine, a nonselective antagonist (Kafka et al., 1977; Newman et al., 1978; Alexander et al., 1978). Although this radioligand does not discriminate between α_1- and α_2-adrenoreceptors, several biochemical (Hoffman et al., 1979) and functional studies (Grant and Scrutton, 1979; Hsu et al., 1979) have suggested that the α-adrenoreceptor on the human platelet is exclusively of the α_2-subtype. Preliminary studies from this laboratory which involved the binding of [^{3}H]clonidine and [^{3}H]yohimbine to platelets indicated that the alpha adrenoreceptor located upon human platelets is an α_2-adrenoreceptor (García-Sevilla et al., 1980). Recently, Motulsky et al. (1980) and Shattil et al. (1981) also reported similar observations that [^{3}H]yohimbine and [^{3}H]clonidine bind to the platelet adrenoreceptor.

α_2-Adrenoreceptors on the human platelet, like those on neurons in the rat brain, appear to become subsensitive after the long-term administration of tricyclic drugs. We have recently reported that the chronic administration of either imipramine or nortriptyline to endogenously depressed patients reduces the number of binding sites for [^{3}H]clonidine on blood platelet membranes (García-Sevilla et al., 1981). This finding suggests that the specific binding of radiolabelled α_2-adrenoreceptor ligands to human platelet membranes might be used to evaluate α_2-adrenoreceptor function in endogenous depression as well as to assess biochemically therapeutic responsiveness. Consequently, the present study was undertaken to directly characterize α_2-adrenoreceptors on human platelet membranes with highly selective radioligands, the agonist [^{3}H]clonidine and the antagonist [^{3}H]yohimbine. In addition, the interaction between antidepressant drugs and platelet α_2-adrenoreceptors was also investigated. The inhibition constants and the nature of the competition against the binding of the tritiated ligands were assessed for a representative group of antidepressant drugs: desipramine, a tricyclic secondary amine; amitriptyline, a tricyclic tertiary amine; and two atypical drugs, mianserin and iprindole.

2. Materials and methods

2.1. Isolation of blood platelets and preparation of membranes

Blood was obtained by venipuncture from healthy male and female volunteers (mean age, 29.8 ± 1.8 years, $n = 18$). Approximately 50 ml of blood was collected in plastic centrifuge tubes containing (8 : 1 v/v) acid-citrate-dextrose (ACD) solution as anticoagulant (National Institutes of Health Formula A: 0.8% citric acid, 2.2% trisodium citrate and 2.45% dextrose). The blood was centrifuged at $160 \times g$ for 10 min (25°C), and the resulting platelet-rich plasma was titrated to pH 6.5 with ACD solution and recentrifuged at $5\,100 \times g$ for 15 min (25°C) to sediment the platelets. The platelet pellet was washed twice with 5 ml of Tyrode buffer (NaCl 137 mM; KCl 2.7 mM; NaH_2PO_4 0.36 mM; $MgCl_2$ 0.10 mM; $NaHCO_3$ 12.0 mM; dextrose 0.56 mM; pH 8.0) and recentrifuged at $5\,100 \times g$ for 15 min. The washed pellet was lysed by homogenization in 2 ml of ice-cold hypotonic buffer (Tris-EDTA, 5 mM; pH 7.5). After centrifugation at $39\,000 \times g$ for 10 min (4°C), the platelet membranes were resuspended in the Tris incubation buffer (Tris-HCl, 50 mM; MgCl 10 mM; pH 7.5) used in the binding assay.

2.2. [^{3}H]Clonidine and [^{3}H]yohimbine binding assay

Total [^{3}H]clonidine or [^{3}H]yohimbine binding was measured in one ml aliquots of the fresh platelet membranes (231 ± 9 μg protein/ml; $n = 83$) which were incubated in duplicate with shaking at 25°C. In the kinetics and drug competition studies, the platelet membranes were incubated for various intervals of time with either [^{3}H]clonidine, 8×10^{-9} M, or with [^{3}H]yohimbine, 4×10^{-9} M. In the equilibrium studies, the platelet membranes were incubated for 20 min with either [^{3}H]cloni-

dine, 10^{-9} M to 6.4×10^{-8} M, or with [^{3}H]yohimbine, 2.5×10^{-10} M to 1.6×10^{-8} M. Nonspecific binding was determined by adding unlabelled clonidine or yohimbine, 10^{-5} M, in addition to [^{3}H]clonidine or [^{3}H]yohimbine, to a second pair of incubates. Specific binding, defined as the difference between total and nonspecific binding, represented $88 \pm 2\%$ (n = 16) and $95 \pm 1\%$ (n = 10) of the total binding at 4×10^{-9} M for [^{3}H]clonidine and [^{3}H]yohimbine, respectively. Incubations were terminated by diluting the sample with 5 ml of Tris incubation buffer (25°C). Membrane bound ^{3}H-ligand was measured by rapid filtration of the diluted sample under vacuum through Whatman GF/C glass fiber filters. After separation of the bound and free ligand, the filters were washed with two 10 ml aliquots of Tris incubation buffer (25°C). Filtration and washing were completed in about 15 sec. The filters were then air dried and placed in glass scintillation vials and counted for radioactivity as descried by Smith et al. (1972). The radioactivity retained by the filters in the absence of tissue (blanks) was proportional to the concentration of radioligand and ranged from 0 to 0.03% of the total radioactivity filtered.

2.3. *Analysis of binding data*

Kinetic studies: the association rate constants (k_{ob}) for the binding of [^{3}H]clonidine and [^{3}H]yohimbine to platelet membranes were calculated from the equation

$$k_{ob} \cdot t = \ln\left[B_{eq}/(B_{eq} - B_t)\right]$$

and the dissociation rate constants (k_2) from

$$k_2 \cdot t = \ln\left[B_t/B_{eq}\right]$$

where B_{eq} is the amount of radioligand specifically bound at equilibrium and B_t represents the amount of radioligand specifically bound at each time interval, t. The slopes of these regression lines, calculated by the method of least squares, equal k_{ob} and k_2, respectively. Second order rate constants (k_1) for both radioligands were calculated from the relation

$$k_1 = k_{ob} - k_2/[\text{radioligand}]$$

where [radioligand] is the concentration of [^{3}H]clonidine (8 nM) or [^{3}H]yohimbine (4 nM) in the binding assay. The kinetically estimated equilibrium dissociation constant (K_D) was obtained from the ratio k_2/k_1.

Equilibrium studies: for platelet membranes from each subject, full saturation curves were determined and the apparent dissociation constant (K_D) and the maximum number of binding sites (B_{max}) for both radioligands were estimated by use of the linear transformation described by Scatchard (1949). In the Scatchard plot, where the amount of ligand bound is plotted against the ratio of the bound to the free ligand, the B_{max} is the x-intercept and the $K_D = -1/\text{slope}$. When nonlinear (biphasic) Scatchard plots for [^{3}H]clonidine were found (40% of the normal control population), the point of inflexion between the high affinity and low affinity binding sites was taken as the point where the highest correlation coefficient for the high affinity binding site was calculated. Hill analyses (1910) were also performed to determine the presence or absence of cooperativity. In the Hill plot, the log [radioligand] is plotted against the ratio $\log[B/(B_{max} - B)]$; where B is the amount of ligand bound and B_{max} the maximum number of binding sites determined by Scatchard analysis. The slope of the regression line equals n_H (Hill number), and K'_D (Hill binding constant) is the x-intercept where $\log[B/(B_{max} - B)] = 0$. The B_{max} is expressed as femtomoles of [^{3}H]clonidine or [^{3}H]yohimbine specifically bound per mg of protein. Protein determinations were performed by the method of Lowry et al. (1951) in which bovine serum albumin was used as the protein standard.

Drug competition studies: the inhibition constants (K_i) for the competing drugs were calculated from the equation (Cheng and Prussoff, 1973): $K_i = IC_{50}/1 + ([\text{radioligand}]/K_D)$; where IC_{50} is the required drug concentration to inhibit the specific binding of [^{3}H]clonidine or [^{3}H]yohimbine by 50%. IC_{50}s were calculated from concentration-response curves by the method of probit analysis described by Goldstein (1964).

2.4. Drugs

[^{3}H]clonidine (specific activity 23.8 Ci/mmol) and [^{3}H]yohimbine (specific activity 81.6 Ci/mmol) were purchased from New England Nuclear, Boston Massachusetts, and stored at −20°C. For the binding assays appropriate amounts of the stock solutions were diluted with glass-distilled water containing 2.5 mM HCl.and 6% ethanol. Other drugs used and their sources included: clonidine hydrochloride (Boehringer, Ingelheim, Germany), yohimbine hydrochloride (Amend Drug & Chemical Co., N.Y.), phenylephrine hydrochloride (Sigma, St. Louis), prazosin hydrochloride (Pfizer Laboratories, N.Y.), amitryptyline hydrochloride (Merck, Sharp & Dohme Research Laboratories, West Point, PA), chlorimipramine hydrochloride (Ciba-Geigy Corp., Summit, N.J.) desipramine hydrochloride (USV Pharmaceuticals, Scarsdale, N.Y.), iprindole hydrochloride (Wyeth Laboratories, Philadelphia, PA) and mianserin hydrochloride (Organon, Oss, Holland). All drugs were dissolved in glass-distilled water.

2.5. Statistics

For the statistical evaluations Student's t-test was used. Correlation coefficients were calculated by the method of least squares. The level of significance was chosen as P = 0.05.

3. Results

3.1. Kinetics of [^{3}H]clonidine and [^{3}H]yohimbine binding to platelet membranes

The specific binding of the agonist [^{3}H]clonidine (8 nM) to fresh platelet membranes was rapid ($t_{1/2}$ = 1.8 min; fig. 1A) and reversible ($t_{1/2}$ = 0.5 min; fig. 1B). At 25°C the association reaction reached equilibrium at 10 min with an initial rate constant (k_{ob}) of 0.391 min^{-1}. When equilibrium was reached, the addition of unlabelled clonidine, 10^{-5} M, resulted in a rapid displacement of the specifically bound [^{3}H]clonidine, which followed first order kinetics with a k_2 of 1.333 min^{-1} (fig. 1B). From the k_2 and k_{ob} a second order rate constant (k_1) of 0.216 min^{-1}·nm^{-1} was calculated (fig. 1A). The equilibrium dissociation constant ($K_D = k_2/k_1$) determined from the kinetics

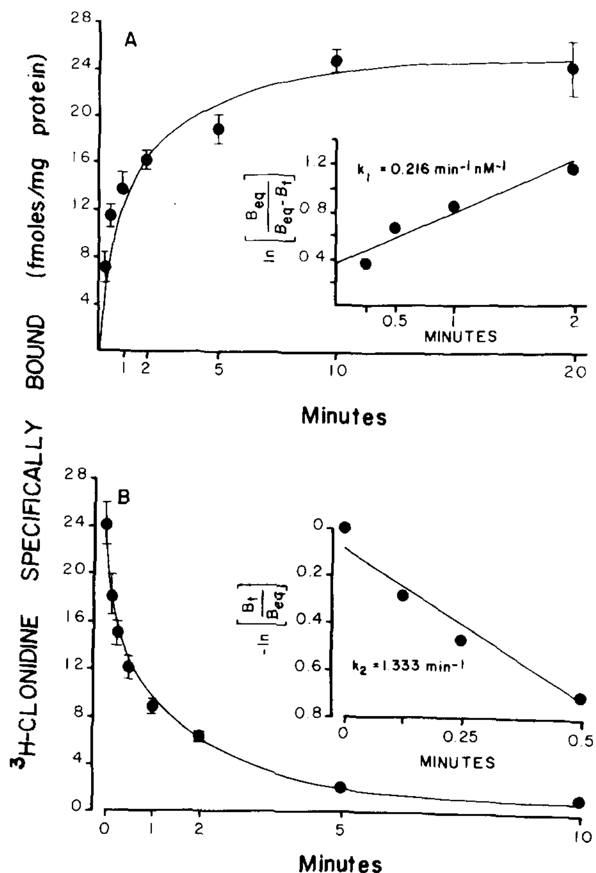

Fig. 1. Kinetic characteristics of the specific binding of [^{3}H]clonidine (8 nM) to human platelet membranes. (A) Time course for the association reaction. *Inset*: pseudo-first order kinetic plot where B_{eq} is the amount (24 fmol/mg protein) of radioligand specifically bound at equilibrium and B_t represents the amount of radioligand specifically bound at each period of time, t. The line, determined by linear regression analysis (r=0.948), has a slope of 0.391 min^{-1} which is equivalent to the observed initial rate constant (k_{ob}). (B) Time course for the dissociation reaction. Platelet membranes were incubated at 25°C for 20 min with [^{3}H]clonidine (8 nM), followed by addition of a large excess of unlabelled clonidine, 10^{-5} M, which corresponds to t=0. The specific binding of [^{3}H]clonidine was determined at various times and plotted as function of time. *Inset*: first-order kinetic plot. The slope of the line, determined by linear regression analysis (r = −0.976) is equivalent to the first order dissociation rate constant (k_2). The second order rate constant (k_1) for the association reaction was calculated from the relation $k_1 = k_{ob} - k_2$/[[^{3}H]clonidine]. Each point is the mean value ±S.E.M. of 3–4 experiments performed in duplicate.

TABLE 1

Inhibition of binding of [^{3}H]clonidine and [^{3}H]yohimbine to platelet membranes by selective adrenergic agonists and antagonists. Binding assay conditions were as in fig. 3. K_i values were calculated from the equation (Cheng and Prussoff, 1973): $K_i = IC_{50}/1 + ([radioligand]/K_D)$. IC_{50} values were determined from the concentration-response curves shown in fig. 3. K_D values for [^{3}H]clonidine ($K_D = 5.0$ nM) and [^{3}H]yohimbine ($K_D = 3.0$ nM) were independently estimated from equilibrium studies. Each value is the mean ± S.E.M. of n determinations performed in triplicate.

Drug	Inhibition of [^{3}H]clonidine binding at 8 nM		Inhibition of [^{3}H]yohimbine binding at 4 nM	
	K_i(nM)	n	K_i(nM)	n
Clonidine	2.1 ± 0.6	3	2.6 ± 1.2	3
Yohimbine	10.6 ± 4.7	4	0.2 ± 0.1	3
Phenylephrine	428.3 ± 34.0	3	243.6 ± 26.7	3
Prazosin	4000	3	5022.0 ± 550.0	3

studies was 6.2 nM, which was in good agreement with the K_D (5.0 nM) estimated from the saturation experiments (see table 2).

The specific binding of the antagonist [^{3}H]yohimbine (4 nM) to platelet membranes was also rapid ($t_{1/2} = 2.7$ min; fig. 2A) and reversible ($t_{1/2} = 8.2$ min; fig. 2B). Equilibrium was reached at 10–20 min with a k_{ob} of 0.261 min^{-1} and a k_1 of 0.044 min^{-1}·nM^{-1} (fig. 2A). At equilibrium conditions, the addition of unlabelled yohimbine, 10^{-5} M, resulted in a slower displacement of the specific binding of [^{3}H]yohimbine when compared to that of [^{3}H]clonidine. However, the dissociation reaction also followed first order kinetics with a k_2 of 0.085 min^{-1} (fig. 2B). The kinetically determined K_D (1.9 nM) was also in good agreement with the K_D (3.0 nM) estimated by equilibrium studies (see table 2).

3.2. Specificity of [^{3}H]clonidine and [^{3}H]yohimbine binding to platelet membranes

The nature of the α_2-adrenoreceptor on the human platelet was further characterized by comparing K_i values for the inhibition of [^{3}H]clonidine and [^{3}H]yohimbine binding by selective adrenergic agonists and antagonists (table 1). Clonidine, a selective α_2-adrenoreceptor agonist, was 100–200 times more potent in displacing the specific binding of both radioligands than phenylephrine, a selective α_1-adrenoreceptor agonist. Moreover, yohimbine, a selective α_2-adrenoreceptor antagonist was 400–2500 times more potent than prazosin, a selective α_1-adrenoreceptor antagonist. Although clonidine and yohimbine were the most potent inhibitors of the binding of the respective ligands, selective adrenergic drugs in

TABLE 2

Parameters of high-affinity binding of [^{3}H]clonidine and [^{3}H]yohimbine to human platelet membranes. The apparent dissociation constants (K_D) and the maximum number of binding sites (B_{max}) were determined by Scatchard analyses of full saturation curves (7 different concentrations of the radioligands each performed in duplicate) for each subject (10 male and 6 female for [^{3}H]clonidine and 5 male and 5 female for [^{3}H]yohimbine). K'_D and n_H represent the Hill dissociation constant and the Hill number respectively, which were calculated as described in the Methods section.

Radioligand	Scatchard analysis		Hill analysis		n
	K_D (nM)	B_{max} (fmol/mg protein)	K'_D (nM)	n_H	
[^{3}H]clonidine	5.0 ± 0.5	35 ± 3	5.2 ± 0.4	1.02 ± 0.03	16
[^{3}H]yohimbine	3.0 ± 0.1 *	188 ± 12 *	3.2 ± 0.2 *	0.98 ± 0.02	10

* $P < 0.001$ (Student's t-test) when compared with [^{3}H]clonidine binding parameters.

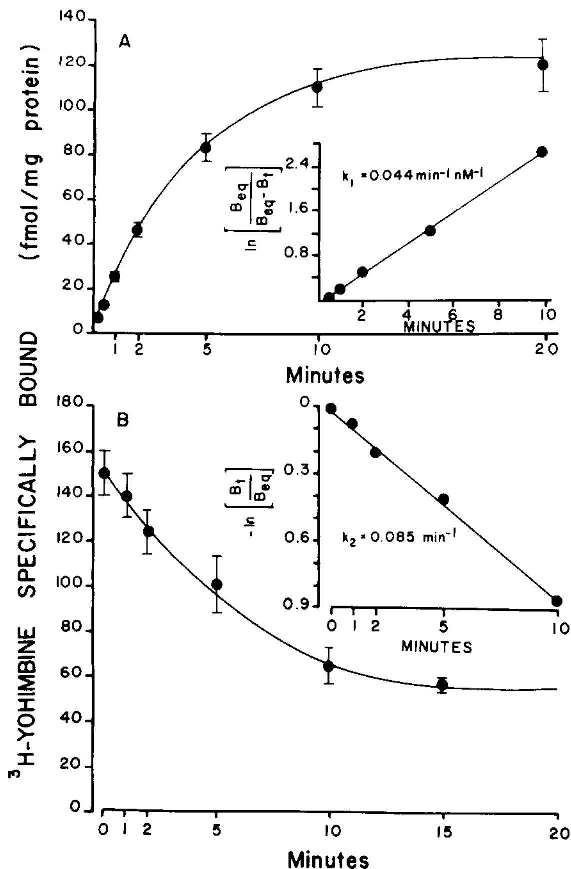

Fig. 2. Kinetic characteristics of the specific binding of [^{3}H]yohimbine (4 nM) to human platelet membranes. (A) Time course for the association reaction. *Inset*: pseudo-first order kinetic plot where B_{eq} is the amount of radioligand specifically bound at equilibrium (118 fmol/mg protein) and B_t represents the amount of radioligand specifically bound at each period of time, t. The line, determined by linear regression analysis (r=0.999), has a slope of 0.261 min^{-1} which is equivalent to the observed initial rate constant (k_{ob}). (B) Time course for the dissociation reaction. Platelet membranes were incubated at 25°C for 20 min with [^{3}H]yohimbine (4 nM), followed by addition of a large excess of unlabelled yohimbine, 10^{-5} M, which corresponds to t = 0. The specific binding of [^{3}H]yohimbine was determined at various times and plotted as function of time. Inset: first-order kinetic plot. The slope of the line, determined by linear regression analysis (r=0.999) is equivalent to the first order dissociation rate constant (k_2). The second order rate constant (k_1) for the association reaction was calculated from the relation $k_1 = k_{ob} - k_2/[[^3H]yohimbine]$. Each point is the mean value ±S.E.M. of 3 experiments performed in duplicate.

competing with either [^{3}H]clonidine or [^{3}H]-yohimbine had an order of potency (clonidine ≃ yohimbine ≫ phenylephrine > prazosin), which

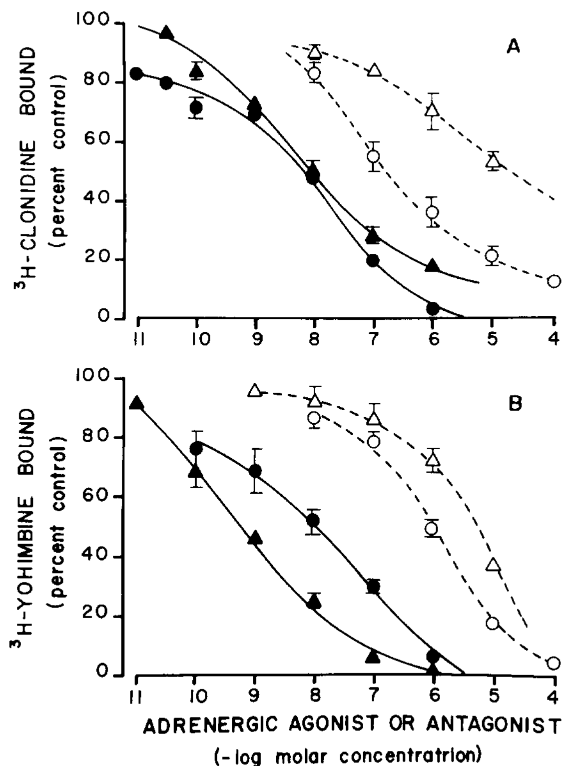

Fig. 3. Inhibition by selective adrenergic drugs of the specific binding of [^{3}H]clonidine (A) and [^{3}H]yohimbine (B) to human platelet membranes. Platelet membranes were incubated in triplicate at 25°C for 20 min with [^{3}H]clonidine (8 nM) or [^{3}H]yohimbine (4 nM) in the absence or presence of various concentrations of the competing drugs. The specific binding was determined as described in the Methods section and plotted as a function of the drug concentration. Each point is the mean value ±S.E.M. of 3–4 experiments. Clonidine (●), yohimbine (▲), phenylephrine (○) and prazosin (△).

is associated with α_2-adrenoreceptors (table 1 and fig. 3).

3.3. Saturability and affinity of [^{3}H]clonidine and [^{3}H]yohimbine binding to platelet membranes

The specific binding of [^{3}H]clonidine and [^{3}H]yohimbine to platelet membranes was a saturable process of high affinity (fig. 4 and table 2). Scatchard analyses of individual saturation curves showed a marked difference in the affinity constants (K_D) and the maximal number of binding sites (B_{max}) for the two radioligands. The K_D value for the high affinity binding of

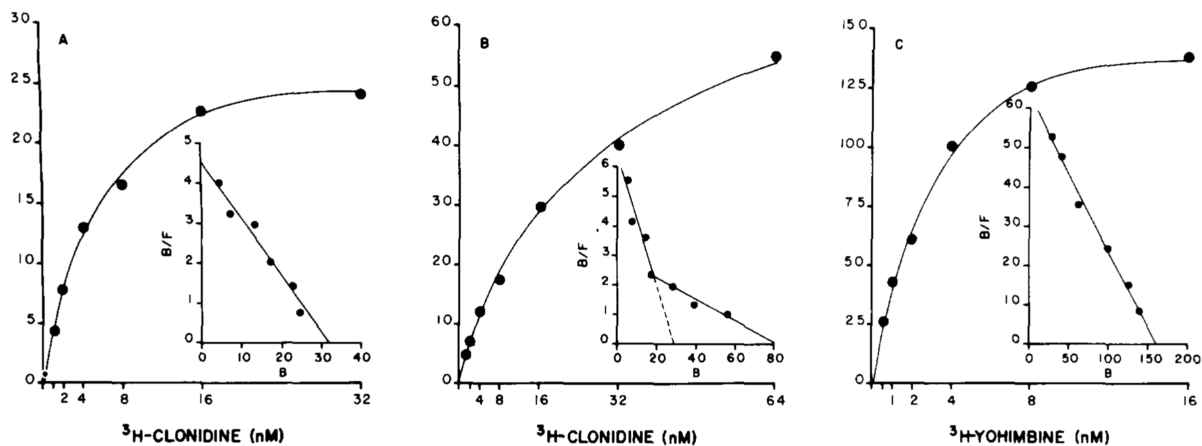

Fig. 4. Specific binding of [^{3}H]clonidine (A and B) and [^{3}H]yohimbine (C) to human platelet membranes as a function of increasing concentrations of the radioligands. *Ordinates*: ^{3}H-ligand specifically bound (fmol/mg protein). *Inset A*: representative Scatchard plot showing the existence of a single population of high affinity sites for [^{3}H]clonidine (K_D = 6.9 nM; B_{max} = 32 fmol/mg protein). *Inset B*: representative Scatchard plot indicating the existence of two binding sites for [^{3}H]clonidine, one of high affinity (K_D = 4.6 nM; B_{max} = 29 fmol/mg protein) and a second site of lower affinity and higher capacity (K_D = 28.1 nM; B_{max} = 81 fmol/mg protein). *Inset C*: representative Scatchard plot showing the existence of a single population of high affinity sites for [^{3}H]yohimbine (K_D = 2.5 nM; B_{max} = 160 mol/mg protein).

[^{3}H]yohimbine was significantly lower than that of [^{3}H]clonidine, and its maximum binding capacity (B_{max}) five times greater (table 2). Hill analyses of [^{3}H]clonidine and [^{3}H]yohimbine binding also gave the same dissociation constants (K'_D) and indicated that there was no cooperativity (n_H not different from 1.0) (table 2). Scatchard analyses of [^{3}H]yohimbine binding also suggested in all cases that only a single population of binding sites was present upon the platelet membranes (fig. 4C). In contrast, Scatchard analyses of [^{3}H]clonidine binding suggested the existence of two sites in about 40% of a normal control population, one of high affinity (table 2 and fig. 4A and B) and a second, noninteracting site of lower affinity and higher capacity (K_D = 18.6 ± 4.5 nM; B_{max} = 77 ± 9 fmol/mg protein; n_H = 1.03 ± 0.08; n = 7; fig. 4B). Neither age nor sex affected the K_D or B_{max} for the tritiated ligands.

3.4. Effects of antidepressant drugs on [^{3}H]clonidine and [^{3}H]yohimbine binding to platelet membranes

The possibility that antidepressant drugs also might compete with [^{3}H]clonidine and [^{3}H]-yohimbine for the platelet α_2-adrenoreceptor was investigated. The presence of iprindole, desipramine, chlorimipramine, amitriptyline or mianserin in the incubation medium at a final concentration of 10^{-5} M markedly decreased the specific binding of both radioligands (46–96%; $P < 0.001$) (fig. 5). When the inhibition constants (K_i) obtained from concentration-response curves were compared, the rank order of potency of the various antidepressant drugs in inhibiting the binding of [^{3}H]clonidine and [^{3}H]yohimbine was similar: mianserin > amitriptyline ≫ iprindole > desipramine; and mianserin > amitriptyline ≫ desipramine > iprindole, respectively (table 3 and fig. 6). Mianserin and amitriptyline were the most potent antidepressants in displacing the specific binding of both radioligands with K_i values in the nanomolar range. Desipramine and iprindole were much less potent with K_i values in the micromolar range. Yet the various antidepressant drugs, with the exception of iprindole, were 2–4 times more potent in inhibiting the specific binding of [^{3}H]yohimbine than that of [^{3}H]clonidine (table 3).

The inhibition of binding induced by the various antidepressant drugs was competitive. The nature of the interaction between the antidepressants and the labelled ligands was evaluated by use of

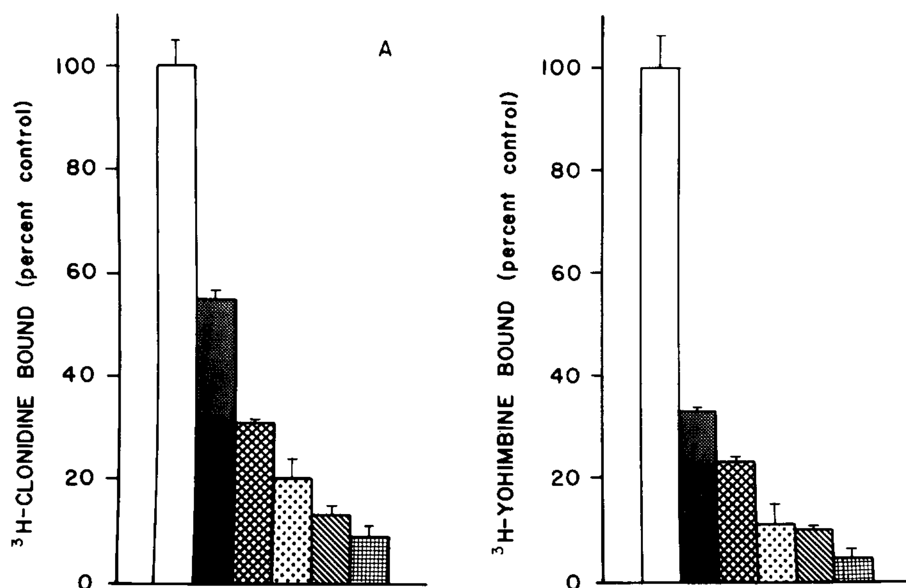

Fig. 5. Effect of various antidepressant drugs on the specific binding of [^{3}H]clonidine and [^{3}H]yohimbine to human platelet membranes. Platelet membranes were incubated in duplicate at 25°C for 20 min with [^{3}H]clonidine (8 nM) or [^{3}H]yohimbine (4 nM) in the absence or presence of the competing drugs (10^{-5} M). The specific binding was determined as described in the Methods section and is expressed as percent of controls. Each bar is the mean value ±S.E.M. of three experiments. Control (□), iprindole (■), desipramine (▩), clomipramine (⊡), amitriptyline (▧) and mianserin (▦).

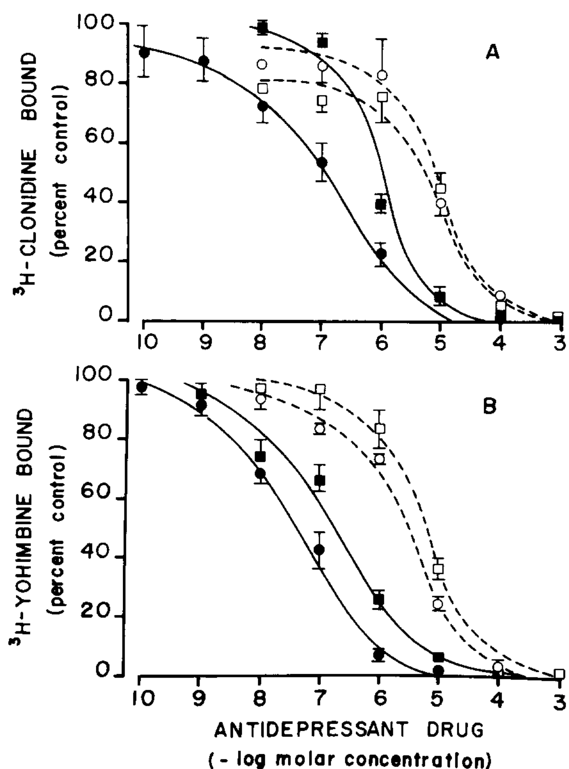

Fig. 6. Inhibition by various antidepressant drugs of the specific binding of [^{3}H]clonidine (A) and [^{3}H]yohimbine (B) to human platelet membranes. Platelet membranes were incubated in triplicate at 25°C for 20 min with [^{3}H]clonidine (8 nM) or [^{3}H]yohimbine (4 nM) in the absence or presence of various concentrations of the competing drugs. The specific binding was determined as described in the Method section and plotted as a function of the drug concentration. Each point is the mean value ±S.E.M. of 3 experiments. Mianserin (●), amitriptyline (■), desipramine (○) and iprindole (□).

concentrations of the antidepressant drugs in the range of their K_is. In the presence of mianserin (10^{-7} M) or iprindole (10^{-5} M) the number of binding sites for [^{3}H]clonidine or [^{3}H]yohimbine was not altered although in each case there was a 2.0–6.0-fold increase in the dissociation constants (fig. 7). Similar results were obtained when amitriptyline was used as the competing drug. In the presence of amitriptyline (10^{-6} M), the K_D for [^{3}H]clonidine was increased 4-fold (control K_D, 6.9 nM; amitriptyline K_D, 27.7 nM) and for [^{3}H]yohimbine was increased 8-fold (control K_D, 3.3 nM; amitriptyline K_D, 27.0 nM). B_{max}s for

TABLE 3

Inhibition of [^{3}H]clonidine and [^{3}H]yohimbine binding to platelet membranes by various antidepressant drugs. Binding assay conditions were as in fig. 6. K_i values were calculated from the equation (Cheng and Prussoff, 1973): $K_i = IC_{50}/1 + ([\text{radioligand}]/K_D)$. IC_{50} values were determined from the concentration-response curves shown in fig. 6. K_D values for [^{3}H]clonidine ($K_D = 5.0$ nM) and [^{3}H]yohimbine ($K_D = 3.0$ nM) were independently estimated from equilibrium studies. Each value is the mean ±S.E.M. of n determinations performed in triplicate.

Drug	Inhibition of [^{3}H]clonidine binding at 8 nM		Inhibition of [^{3}H]yohimbine binding at 4 nM	
	K_i(nM)	n	K_i(nM)	n
Mianserin	36± 9	3	19± 4	3
Amitriptyline	318± 23	3	76± 6	3
Desipramine	2558±298	3	961± 82	3
Iprindole	1641±314	3	2096±774	3

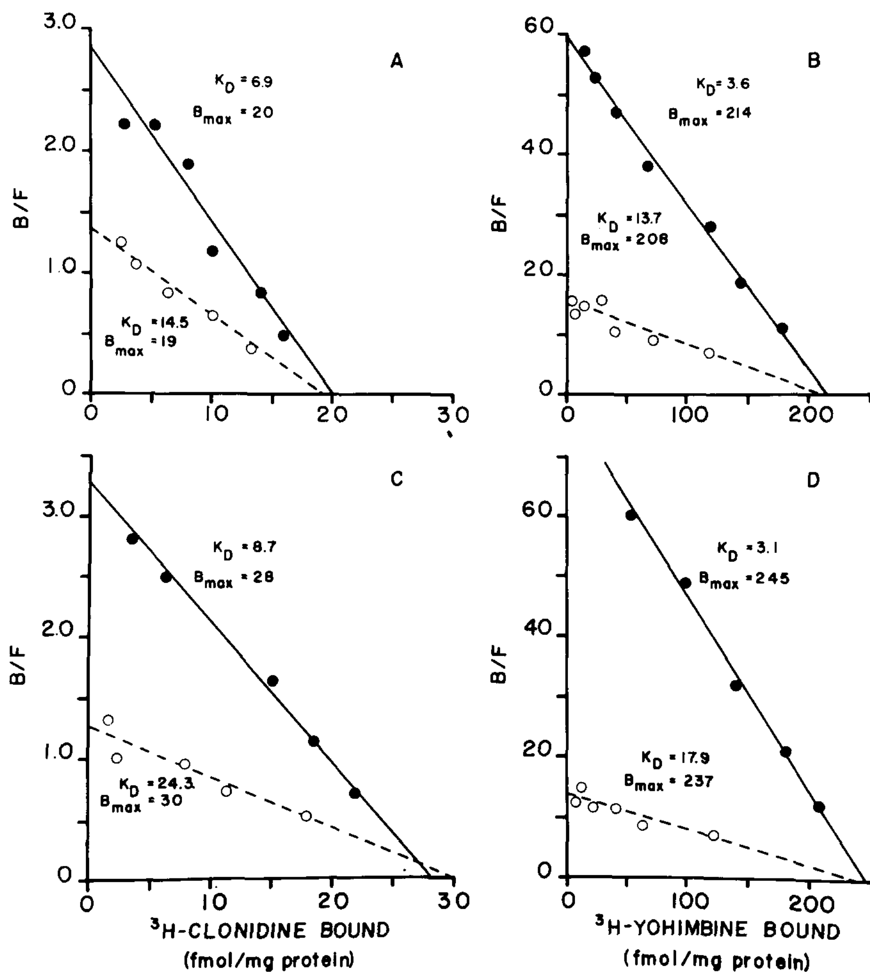

Fig. 7. Representative Scatchard plots for the competitive inhibition induced by mianserin and iprindole upon the specific binding of [^{3}H]clonidine and [^{3}H]yohimbine to human platelet membranes. Platelet membranes were incubated in duplicate at 25°C for 20 min with 5–7 different concentrations of [^{3}H]clonidine or [^{3}H]yohimbine in the absence of presence of mianserine or iprindole. The specific binding was determined as described in the Methods section and plotted as a function of B/F (bound ligand/free ligand). Each figure represents a different competition study performed in the same subject. (A) Inhibition of [^{3}H]clonidine binding by mianserine (10^{-7} M). Control (●); mianserin (○). (B) Inhibition of ^{3}H-yohimbine binding by mianserin (10^{-7} M). Control (●); mianserin (○). (C) Inhibition of [^{3}H]clonidine binding by iprindole (10^{-5} M). Control (●); iprindole (○). (D) Inhibition of [^{3}H]yohimbine binding by iprindole (10^{-5} M). Control (●); iprindole (○).

neither ligand were significantly altered by amitriptyline. Desipramine was much less effective in displacing the labelled ligands than were the other antidepressant drugs. In the presence of desipramine (10^{-6} M), the K_D for [^{3}H]clonidine was increased 1.5-fold (control K_D, 4.8 nM; desipramine K_D, 7.0 nM) and for [^{3}H]yohimbine was increased 3-fold (control K_D, 2.5 nM; desipramine K_D, 7.2 nM). B_{max}s for neither ligand were significantly altered by desipramine.

4. Discussion

The binding characteristics of both [^{3}H]clonidine and [^{3}H]yohimbine to human platelet membranes satisfy criteria which are required for the identification of a physiological α_2-adrenoreceptor, namely the binding of both radioligands is rapid, reversible, specific, saturable and of high affinity. Clonidine is an agonist with high affinity for the α_2-adrenoreceptor and yohimbine is an antagonist with high affinity for the α_2-adrenoreceptor (Farnebo and Hamberger, 1971; Starke et al., 1975a; Starke, 1977). The kinetic characteristics of the binding of both [^{3}H]clonidine and [^{3}H]-yohimbine to fresh platelet membranes are very similar, except that the dissociation of [^{3}H]-yohimbine from the receptor was slower than that of [^{3}H]clonidine, as would be expected for an antagonist. A similarly slow dissociation reaction from human platelet membranes has been reported for [^{3}H]dihydroergocryptine, a nonselective α-adrenoreceptor antagonist (Newman et al., 1978; Alexander et al., 1978). Competition experiments with selective adrenergic drugs demonstrated further the specificity of [^{3}H]clonidine and [^{3}H]yohimbine for the α_2-adrenoreceptor. The rank order of potency obtained (clonidine $\simeq$ yohimbine $\gg$ phenylephrine $>$ prazosin) was that typically expected for drugs which act upon the α_2-adrenoreceptor. Phenylephrine is an agonist with high affinity for the α_1-adrenoreceptor (Starke et al., 1975b) and prazosin is an antagonist with high affinity for the α_1-adrenoreceptor (Cambridge et al., 1977).

The specific binding of [^{3}H]clonidine ana [^{3}H]yohimbine to human platelet membranes was saturable (limited number of binding sites) and of high affinity (dissociation constants in the nanomolar range). The affinity constant (K_D) and the maximum binding capacity (B_{max}) for [^{3}H]yohimbine (table 2) were practically identical to those reported for the nonselective α_2-adrenoreceptor antagonist [^{3}H]dihydroergocryptine (Newman et al., 1978). A similar number of binding sites for both radioligands indicates that [^{3}H]yohimbine labels the entire population of α-adrenoreceptors in the human platelet and confirms by selective labelling the previous study by Hoffman et al. (1979) which suggested that the adrenoreceptor is exclusively of the α_2-subtype.

In human platelets the number of α_2-binding sites labelled by [^{3}H]yohimbine was five times greater than that quantitated by [^{3}H]clonidine (table 2). Since both radioligands have marked selectivity for the α_2-adrenoreceptor, a similar number of binding sites for [^{3}H]clonidine and [^{3}H]yohimbine would have been expected if they interact with the same receptor. One possibility is that [^{3}H]clonidine and [^{3}H]yohimbine label different subpopulations of α_2-adrenoreceptors. This appears to be unlikely since the order of potency of selective adrenergic drugs (table 1 and fig. 3) and various antidepressant drugs (table 3 and fig. 6) in competing with both radioligands was similar. Moreover, there was a significant linear correlation ($r = 0.970$, $P < 0.001$) between the inhibition constants (K_i) of selective adrenergic drugs (with the exception of yohimbine) and the various antidepressant drugs in competing for the binding of [^{3}H]clonidine and [^{3}H]yohimbine (fig. 8). This correlation suggests that both radioligands label the same α_2-adrenoreceptor on the human platelet.

If [^{3}H]clonidine and [^{3}H]yohimbine recognize the same receptor, an intrinsic property of the drugs might account for the differences in B_{max} for the two radioligands. It has been suggested that clonidine behaves as a partial agonist on α_2-adrenoreceptors located on noradrenergic nerve terminals (Medgett et al., 1978; Sullivan and Drew, 1980) as well as on those located on human platelets (Newman et al., 1978; Grant and Scrutton, 1979, 1980; Hsu et al., 1979). Therefore, the lower maximum binding capacity of [^{3}H]clonidine might

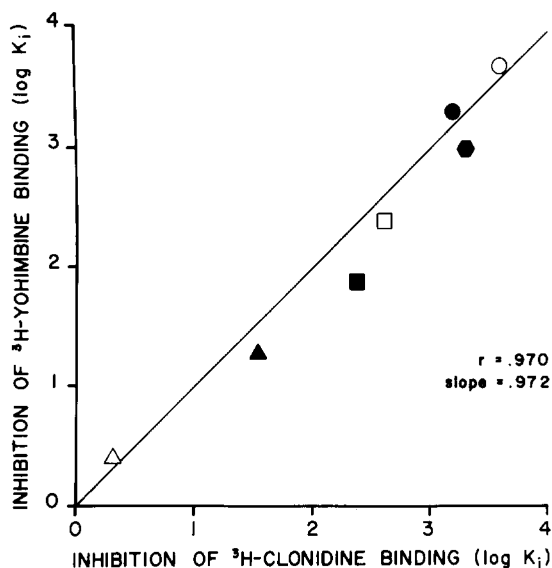

Fig. 8. Correlation between the inhibition constants (K_i) of various drugs in competing for [^{3}H]clonidine and [^{3}H]yohimbine binding to human platelet membranes. The log K_i were calculated from the K_i values shown in tables 1 and 3. The K_i value for prazosin against [^{3}H]clonidine binding was taken as 4000 nM. The K_i values for yohimbine were excluded from the correlation due to the high potency displayed against its homologous tritiated ligand. The line represents the regression of the perfect theoretical correlation (slope = 1.000, r = 1.000). Clonidine (△), mianserin (▲), amitriptyline (■), phenylephrine (□), iprindole (●), desipramine (●) and prazosin (○).

be related to an ability of the drug to detect only a portion (subunit) of the α_2-adrenoreceptor.

Scatchard analyses of the binding data for [^{3}H]yohimbine indicated that only one class of noninteracting binding sites was present on human platelets (fig. 4C). In contrast, two classes of noninteracting binding sites for [^{3}H]clonidine were detected in a substantial proportion (40%) of a normal control population (fig. 4A,B). It is not known at present the nature and relevance of a second, low affinity binding site for [^{3}H]clonidine on human platelets. It should be mentioned, however, that the same subjects who had two binding sites on platelets for [^{3}H]clonidine only had one binding site for [^{3}H]yohimbine.

Recently, the long-term administration of various tricyclic antidepressant drugs to endogenously depressed patients was found to be associated with a decrease in the number of binding sites for [^{3}H]clonidine on platelet membranes (García-Sevilla et al., 1981). Since the observed inhibition of the binding of [^{3}H]clonidine induced by antidepressant drugs might be related to their antagonistic properties upon the α_2-adrenoreceptor (Baumann and Maître, 1977; Fludder and Leonard, 1979; Cavero et al., 1980; Tang and Seeman, 1980; Smith et al., 1981), the nature of the drug competition was studied in vitro. Only high concentrations of desipramine and iprindole (K_i values in the micromolar range) inhibited the specific binding of [^{3}H]clonidine and [^{3}H]yohimbine to platelet membranes. In contrast, mianserin and amitriptyline were fairly potent in displacing the specific binding of both radioligands with K_i values in the nanomolar range (table 3). Moreover, all antidepressant drugs, with the exception of iprindole, were more potent in inhibiting the binding of the antagonist [^{3}H]yohimbine than that of the agonist [^{3}H]clonidine, which might further support their α_2-adrenoreceptor blocking properties (table 3).

The changes in the platelet α_2-adrenoreceptor which are seen after long-term administration of antidepressant drugs do not appear to result from a direct inhibition of the platelet receptor by the drug in the plasma. Plasma concentrations of amitriptyline and mianserin, but not of desipramine and iprindole, similar to those required for the inhibition of the binding of [^{3}H]clonidine or [^{3}H]yohimbine to platelet membranes, are most probably reached during their long-term administration to psychiatric patients (Ziegler et al., 1976; Montgomery et al., 1978; Perry et al., 1978). However, the inhibition of the binding of [^{3}H]clonidine and [^{3}H]yohimbine to platelet membranes induced by all antidepressant drugs was competitive (fig. 7) in that the K_D for binding was increased whereas the B_{max} remained unchanged. These results are in marked contrast to those obtained after the chronic administration of antidepressant drugs to psychiatric patients where the inhibition of the binding of [^{3}H]clonidine was associated with a decrease in both the number of binding sites (lower B_{max}) and the dissociation constant for the radioligand (lower K_D) (García-Sevilla et al., 1981).

α_2-Adrenoreceptors on human platelets appear to be very similar to those found in the mammalian brain. Although there are slight differences

in relative potencies, the rank order of potency of selective adrenergic drugs in inhibiting the binding of [^{3}H]clonidine to human platelets (clonidine > yohimbine ≫ phenylephrine > prazosin) (present study) was similar to that found with the same radioligand in the rat cerebral cortex (U'Prichard et al., 1979). Moreover, the order of potency of various antidepressant drugs in competing with [^{3}H]clonidine for the α_2-adrenoreceptor on human platelet membranes (mianserin > amitriptyline ≫ iprindole > desipramine) (present study) was also in close agreement with that reported for the same radioligand in the calf frontal cortex (mianserin > amitriptyline ≫ desipramine > iprindole) (Tang and Seeman, 1980). More remarkably, the parameters of high-affinity [^{3}H]clonidine binding to human platelet membranes (K_D = 5.0 nM; B_{max} = 35 fmol/mg protein) (present study) were comparable to those described in different areas of the human brain (K_D = 0.5–2.0 nM; B_{max} = 10–51 fmol/mg protein) (Weinreich et al., 1980). These pharmacological and biochemical similarities most probably indicate that a related, if not the same, α_2-adrenoreceptor entity is present in the brain and in the blood platelet.

We have recently reported that α_2-adrenoreceptors on human platelets (García-Sevilla et al., 1981), like those on neurons in the rat brain (Smith et al., 1981) also become subsensitive after the long-term administration of tricyclic antidepressant drugs. It therefore appears that changes in platelet α_2-adrenoreceptors during tricyclic antidepressant drug treatment reflect those occuring in the brain. The specific binding of radiolabelled α_2-adrenoreceptor ligands to human platelet membranes might be a new and useful tool for the study of α_2-adrenoreceptor abnormalities in endogenous depression and a simple laboratory test to monitor therapeutic responsiveness.

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