Identification of α-Adrenergic Receptors in Human Platelets by [^{3}H]Dihydroergocryptine Binding

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ABSTRACT Binding of [^{3}H]dihydroergocryptine to platelet lysates appears to have all the characteristics of binding to α-adrenergic receptors. At 25°C binding reaches equilibrium within 20 min and is reversible upon addition of excess phentolamine. Binding is saturable with 183±22 fmol of [^{3}H]dihydroergocryptine bound per mg of protein at saturation, corresponding to 220±26 sites per platelet. Kinetic and equilibrium studies indicate the dissociation constant of [^{3}H]dihydroergocryptine for the receptors is 1–3 nM. The specificity of the binding sites is typical of an α-adrenergic receptor. Catecholamine agonists compete for occupancy of the [^{3}H]dihydroergocryptine binding sites with an order of potency (−)epinephrine> (−)norepinephrine≫ (−)isoproterenol. Stereospecificity was demonstrated inasmuch as the (+)isomers of epinephrine and norepinephrine were 10–20-fold less potent than the (−)isomers. The potent α-adrenergic antagonists phentolamine, phenoxybenzamine, and yohimbine competed potently for the sites, whereas β-antagonists such as propranolol and dichlorisoproterenol were quite weak. Dopamine and serotonin competed only at high concentrations (0.1 mM).

The [^{3}H]dihydroergocryptine binding sites could also be demonstrated in intact platelets where they displayed comparable specificity, stereospecificity, and saturability. Saturation binding studies with the intact platelets indicated 220±45 receptors per platelet, in good agreement with the value derived from studies with platelet lysates. Ability of α-adrenergic agonists to inhibit adenylate cyclase and of α-adrenergic antagonists to antagonize this inhibitory effect directly paralleled ability to interact with the [^{3}H]dihydroergocryptine binding sites. These data demonstrate the feasibility of directly studying α-adrenergic receptor binding sites in human platelets with [^{3}H]dihydroergocryptine.

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INTRODUCTION

The endogenous catecholamines epinephrine and norepinephrine exert a variety of regulatory functions on human physiological processes. The initial sites of action of catecholamines seem to be at membrane adrenergic receptors. Based on the relative order of potencies of a series of adrenergic agonists, Ahlquist proposed that the effects of catecholamines were mediated by two distinct types of adrenergic receptors, termed α- and β-adrenergic receptors (1). β-Adrenergic responses (isoproterenol> epinephrine> norepinephrine) which are specifically antagonized by propranolol are typified by relaxation of smooth muscle and stimulation of cardiac contractility. α-Adrenergic responses (epinephrine> norepinephrine> isoproterenol) are potently antagonized by phentolamine and are typified by contraction of vascular and uterine smooth muscle.

Recent studies have shown that epinephrine and norepinephrine induce human platelet aggregation whereas isoproterenol does not (2–4). This aggregation is blocked by the α-antagonist phentolamine (2–4). Epinephrine and norepinephrine also cause a rapid decrease in prostaglandin E_1-(PGE_1)[1] enhanced cyclic AMP (cAMP) levels in platelets and produce immediate decreases in platelet adenylate cyclase activity (5–11). These responses are also blocked by the α-adrenergic blocker phentolamine. Thus, there is evidence that these adrenergic effects on platelets are mediated by α-adrenergic receptors.

The development of radioligand binding methods for the direct study of hormone and drug receptors over the past few years has facilitated the investigation of the properties and regulation of these receptors. Applied to human cell types, these methods provide an approach to the direct study of abnormalities of receptor function in various disease states (12, 13).

Recently, Williams et al. described a ligand, [^{3}H] dihydroergocryptine, which could be used to identify

[1] *Abbreviations used in this paper*: cAMP, cyclic AMP; PGE_1, prostaglandin E_1.

α-adrenergic receptors in uterine smooth muscle (14). In the present studies we report the successful identification and characterization of α-adrenergic receptors in human platelets with [^{3}H]dihydroergocryptine. Binding of [^{3}H]dihydroergocryptine to human platelet lysates has all the characteristics of specificity, stereoselectivity, saturability, and reversibility which would be expected of binding to a physiological α-adrenergic receptor. α-Receptor binding is also demonstrated in intact platelets. The specificity of the binding data is also correlated with an α-adrenergic response in the platelet lysates inhibition of PGE_1-stimulated adenylate cyclase.

METHODS

Pharmacological agents. [^{3}H]Dihydroergocryptine was used as the α-adrenergic radioligand for this study. It has a specific activity of 23.0 Ci/mmol and has biologic activity and chromatographic properties identical to native dihydroergocryptine (14). Other compounds used in this study were from the following sources: Sigma Chemical Co., St. Louis, Mo.—(−)epinephrine bitartrate, (−)norepinephrine bitartrate, (−)isoproterenol bitartrate, dopamine hydrochloride, dihydroxyphenylalanine, dihydroxymandelic acid, (±)normetanephrine, dihydroergotamine, ergotamine tartrate, ergocryptine, (−)phenylephrine hydrochloride, and (±)propranolol; Winthrop Laboratories, New York—phenylephrine hydrochloride (used only in certain experiments); Sandoz Pharmaceuticals, E. Hanover, N. J.—dihydroergocryptine methane sulfonate; Sterling Drug, Inc., New York—(+)epinephrine bitartrate and (+)norepinephrine bitartrate; Ayerst Laboratories, New York—practolol hydrochloride and (+)butaclamol; Eli Lilly and Co., Indianapolis, Ind.—(±)dichlorisoproterenol; CIBA Pharmaceutical Co., Div. of CIBA-Geigy Corp., Summit, N. J.—phentolamine hydrochloride; McNeil Laboratories, Inc., Fort Washington, Pa.—dibozane and haloperidol; ICN Nutritional Biochemicals Div., Cleveland, Ohio—yohimbine hydrochloride; Boehringer Ingelheim Ltd., Elmsford, N. Y.—clonidine hydrochloride; Burroughs Wellcome Co., Research Triangle Park, N. C.—methoxamine hydrochloride; and Smith Kline & French Laboratories, Div. of Smith Kline Corp., Philadelphia, Pa.—phenoxy-benzamine hydrochloride.

Preparation of platelets and platelet lysates. 100–200 cm^3 of blood were taken from peripheral veins of healthy human volunteers. The first 5 cm^3 of blood was discarded, and the remainder was collected with 3.2% sodium citrate as anticoagulant. The blood was centrifuged at 380 *g* for 10 min. The resulting platelet-rich plasma was centrifuged at 16,000 *g* for 10 min at 25°C. The resulting platelet pellet was resuspended in "buffer I" (Tris-HCl, 0.05 M, pH 7.35, NaCl 0.15 M, EDTA 0.02M) and then recentrifuged at 16,000 *g* for 10 min, resuspended in buffer I, and recentrifuged at 16,000 *g* for 10 min at 25°C. The washed platelets were resuspended in buffer I at a concentration of $1–2 \times 10^9$ platelets/ml for use in binding assays or were resuspended in "lysing buffer" (Tris HCl 0.005M, pH 7.5, EDTA 0.005M) at 4°C for 1 min for preparation of platelet lysates. No significant contaminating leukocytes were found in stained preparations. This suspension was homogenized for 20 strokes with a motor-driven teflon-tipped pestle (controlled by a Powerstat rheostat (Fisher Scientific Co., Pittsburgh, Pa.) at a setting of 120 (−) pestle clearance 0.1–0.5 mm) and then centrifuged at 39,000 *g* for 10 min at 4°C. The final pellet was resuspended in "incubation buffer" (Tris-HCl 0.05 M, pH 7.5, EDTA 0.005 M) for use in the binding assay.

Whole platelet and lysate preparations were examined by phase-contrast and interference-contrast microscopy at ×1000 magnification as well as by electron microscopy. The platelet preparations appeared intact. The lysate preparations show lysis of some platelets with areas of cell debris including scattered cytoplasmic granules and granule-free membrane fragments. Some apparently unlysed platelets were also seen, but these were markedly swollen.

[^{3}H]*Dihydroergocryptine binding assay.* [^{3}H]Dihydroergocryptine (5 nM unless otherwise specified) and platelet lysates (2.0 mg/ml unless otherwise specified) or intact platelets ($1–2 \times 10^9$/ml) were incubated at 25°C with shaking for 18 min in a total volume of 150 μl of incubation buffer for platelet lysates, or buffer I for intact platelets. In competition experiments, varying concentrations of agonists or antagonists were added to the incubation as indicated. Incubations were terminated by rapidly diluting the total volume with 2 ml of incubation buffer or buffer I at 25°C followed by rapid filtration through Whatman GFC glass fiber filters (Whatman, Inc., Clifton, N. J.). Filters were rapidly washed with 20 ml of incubation buffer. After drying, filters were counted in a Triton-toluene scintillation mixture at an efficiency of 40–50%.

"Nonspecific" binding was defined as binding which was not displaced by a high concentration of 10 μM phentolamine, a potent α-adrenergic antagonist, or 1 mM epinephrine, an α-adrenergic agonist. In platelet lysates, 1 mM epinephrine and 10 μM phentolamine inhibited binding to the same extent, and higher concentration of phentolamine did not inhibit binding further. In intact platelets, 10 μM phentolamine inhibited binding slightly, but consistently to a greater extent (~ 20%), than 1 mM epinephrine. Accordingly in studies with intact platelets we have used 1 mM epinephrine to determine nonspecific binding, assuming that all true α-adrenergic receptors should be accessible to epinephrine as well as phentolamine. "Specific" binding, defined as total binding minus nonspecific binding is shown in all figures and tables. Specific binding was generally 55–70% of the [^{3}H]dihydroergocryptine bound in the platelet lysates and 30–40% in the intact platelets. In competition experiments with [^{3}H]dihydroergocryptine present at ~5 nM, specific binding was generally ~110 fmol/mg protein in the platelet lysates.

To assess the integrity of the [^{3}H]dihydroergocryptine the following experiment was performed. Binding incubations were performed in the usual manner. The membranes were then collected by centrifugation. Radioactivity from the supernate and that extracted from the pellet with ethanol was then chromatographed on thin layer plates as previously described in the solvent system chloroform:ethanol:acetic acid:9:5:1 (14). Both the bound and free [^{3}H]dihydroergocryptine displayed R_fs identical to native authentic material.

Protein was determined by the method of Lowry et. al. (15).

Adenylate cyclase assays. Adenylate cyclase assays of platelet lysates were performed by methods comparable to those previously described from this laboratory (16). Assay volume was 50 μl containing 30 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 0.1 mM cAMP, 1.5 mM ATP [α-^{32}P]ATP ($1–2 \times 10^6$ cpm), 5 mM phosphoenolpyruvate, pyruvate kinase (40 μg/ml), and myokinase (20 μg/ml). Incubations were for 10 min at 37°C and were stopped by addition of 1 ml of a solution containing [^{3}H]cAMP (15,000 cpm/ml), 100 μg ATP, and 50 μg cAMP. [^{32}P]cAMP that was formed was isolated by the method of Saloman et al. (17). In separate experiments, it was demonstrated that the assay was linear with respect to protein and time over the time and concentration ranges used in these studies, and that the stopping solution in fact stopped the enzymatic reaction.

For the routine assay of α-adrenergic effects on platelet adenylate cyclase, the enzyme was stimulated by 10 μM PGE_1. The ability of α-adrenergic agonists such as epinephrine to inhibit the PGE_1-stimulated enzyme was then measured. Inasmuch as has been noted previously (10), platelet lysate adenylate cyclase is stimulated via a β-adrenergic receptor, all assays also contained 10 μM (±)propranolol to block β-adrenergic receptors. In general, PGE_1-stimulated adenylate cyclase activity in the platelet lysates was 869±39 pmol cAMP generated/min per mg protein and was maximally reduced to 462 ±24 pmol/min per mg by 1 mM epinephrine (n = 10 experiments).

Platelet aggregation. Platelet-rich plasma was prepared as described above. Platelet aggregation was initiated by addition of (−)epinephrine to a final concentration of 30 μM in a volume of 0.5 ml of stirred platelet rich plasma at 37°C. Aggregation was followed using a platelet aggregation profiler (PAP-3, Bio/Data Corp., Willow Grove, Pa.)

RESULTS

Kinetic characteristics of [^3H]dihydroergocryptine binding to platelet lysates. The time-course of specific binding of [^{3}H]dihydroergocryptine to human platelet lysates at 25°C is shown in Fig. 1. Binding was reversible upon addition of excess phentolamine (Fig. 2). Assuming the binding reaction to be a simple bimolecular process, the forward rate constant (k_1) is calculated to be $1.8 \times 10^7 M^{-1}min^{-1}$. The first order reverse rate constant (dissociation rate constant) is 0.018 min^{-1}. The ratio k_2/k_1 (1 nM) provides a kinetically derived estimate of the dissociation constant of [^{3}H] dihydroergocryptine for the binding sites. This is in reasonable agreement with the K_d (3.1 nM) determined

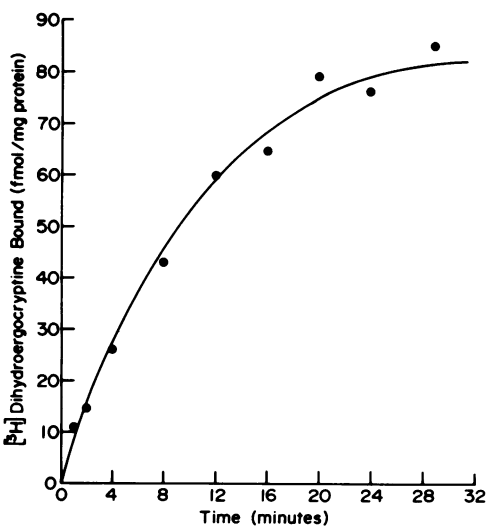

FIGURE 1 [^{3}H]Dihydroergocryptine binding to human platelet lysates as a function of time. [^{3}H]Dihydroergocryptine (3.9 nM) was incubated with platelet lysates (~2 mg/ml) for the indicated times, and specific binding was determined as described in Methods. Each value is the mean of duplicate determinations.

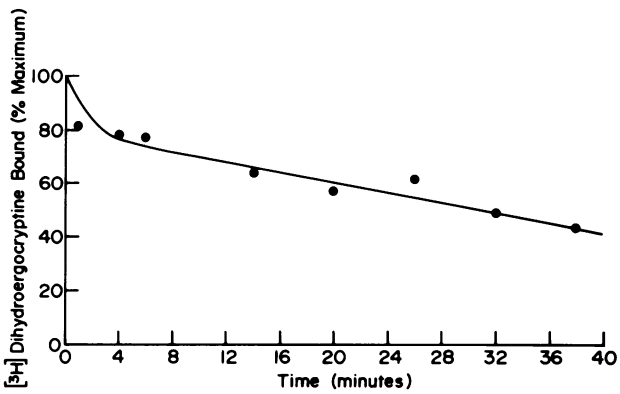

FIGURE 2 Reversibility of [^{3}H]dihydroergocryptine binding to platelet lysates. Lysates were incubated with [^{3}H]dihydroergocryptine (5.5 nM) at 25°C for 16 min after which a large excess of phentolamine (10 μM) was added. The time of phentolamine addition is defined in this figure as time = 0. At the indicated times, specific [^{3}H]dihydroergocryptine binding was determined. Maximum binding is defined as the amount of binding just before the addition of phentolamine at time = 0. Each value is the mean of duplicate determinations.

by equilibrium studies (see below). It should be noted, however, that the reverse kinetic experiment shows an initial rapid loss of ~20% of the binding followed by a slower decay. The significance of this initial rapidly reversing component of dissociation is not known.

Equilibrium characteristics of [^3H]dihydroergocryptine binding to platelet lysates. Binding was a saturable process with 183±22 fmol (mean±SEM) of [^{3}H]dihydroergocryptine bound per mg of protein at saturation (Fig. 3). Half-maximal saturation occurred at 3.1 nM [^{3}H]dihydroergocryptine providing an estimate of the equilibrium dissociation constant, K_d of [^{3}H]dihydroergocryptine for the binding sites. Inasmuch as we found that 5×10^8 platelets provide 1 mg of lysate protein, the saturation value of 183 fmol/mg corresponds to 220±26 receptors per platelet.

Specificity of the [^3H]dihydroergocryptine binding sites in platelet lysates. Adrenergic agonists competed for the [^{3}H]dihydroergocryptine binding sites with an order of potency, (−)epinephrine > (−)norepinephrine > (−)isoproterenol (Fig. 4). The α-adrenergic agonists had high affinity for the binding sites, with K_d values of 0.26 μM and 0.85 μM for (−)epinephrine and (−)norepinephrine, respectively (Table I). The binding sites exhibited stereospecificity with K_d values for the (+)isomers of epinephrine and norepinephrine 8.5- and 20-fold higher than the values for the corresponding (−)isomers.

α-Adrenergic antagonists potently competed for the [^{3}H]dihydroergocryptine binding sites. Phentolamine, a potent α-antagonist, competed with a K_d of 0.014 μM (Table I). Other α-antagonists such as phenoxybenza-

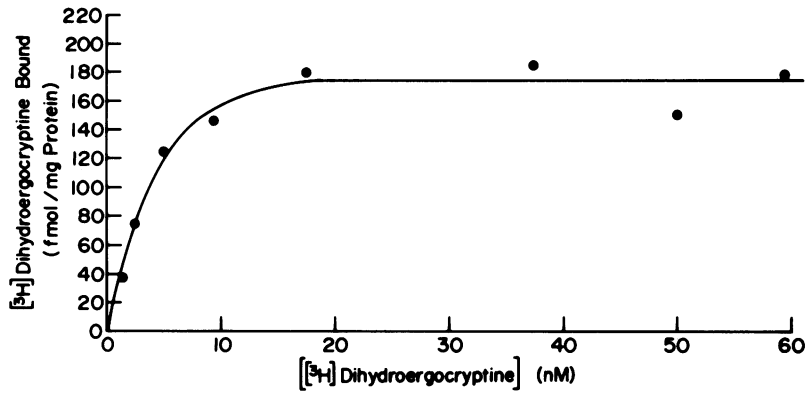

FIGURE 3 Specific binding of [^{3}H]dihydroergocryptine to human platelet lysates as a function of [^{3}H]dihydroergocryptine concentration. Human platelet lysates were incubated with indicated concentrations of [^{3}H]dihydroergocryptine, and binding was determined as described in Methods. Each value is the mean of duplicate determinations from four separate experiments.

mine, yohimbine, and dibozane also inhibited the [^{3}H]-dihydroergocryptine binding (Table I).

In contrast, β-adrenergic antagonists such as propranolol, dichlorisoproterenol, and practolol competed only at high concentrations for the binding sites. Compounds that are devoid of α-adrenergic physiologic effects, but which are structurally related to catecholamines, such as DOPA, dihydroxymandelic acid, normetanephrine, and catechol, did not compete for the [^{3}H]dihydroergocryptine binding sites. Dihydroergocryptine itself competed potently for the binding sites and was five times more potent than the parent compound ergocryptine (Table I). Several other ergot alkaloids such as ergotamine and dihydroergotamine were also potent in competing for binding (Table I).

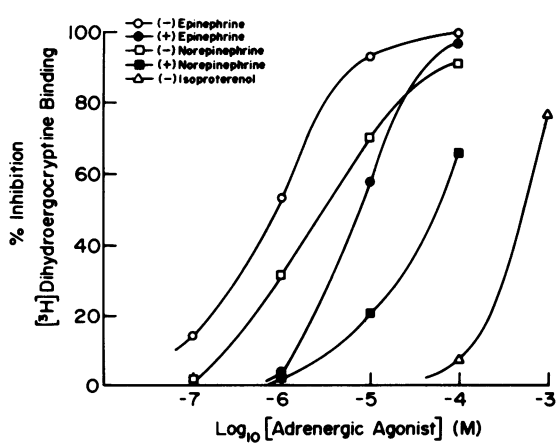

FIGURE 4 Inhibition of [^{3}H]dihydroergocryptine binding by adrenergic agonists. Platelet lysates were incubated with [^{3}H]dihydroergocryptine (5.5 nM) in the absence and presence of the indicated agonists, and specific binding was determined. Each value is the mean of duplicate determinations from at least two separate experiments.

Dopamine had a K_d of 3.1 μM consistent with its known α-adrenergic activity. Serotonin had a low affinity for the binding sites with a K_d of 24.0 μM. When added to maximally effective concentrations of phentolamine (10 μM), high concentrations of dopamine and serotonin did not displace additional [^{3}H]dihydroergocryptine. The α-agonist clonidine was about 10 times more potent than (−)epinephrine in inhibiting [^{3}H]-dihydroergocryptine binding (Table I).

The dopaminergic antagonists haloperidol and (+)-butaclamol inhibited binding but at considerably higher concentrations than the classical α-blockers. This result is consistent with the known weak α-adrenergic antagonist activity of these compounds (18).

K_d values for a number of agents as assessed by [^{3}H]dihydroergocryptine binding are summarized in Table I.

[^{3}H]Dihydroergocryptine binding to intact platelets. [^{3}H]Dihydroergocryptine also bound to intact platelets. The kinetics of binding were very comparable to those observed with lysates. At 23°C, binding reached steady state by 20 min. Binding was also fully reversible with a $t_{1/2}$ of 30′ (cf. Fig. 2). Fig. 5 demonstrates a saturation plot for specific binding of [^{3}H]dihydroergocryptine to intact platelets. The apparent K_d (~4 nM) is in excellent agreement with that determined in the platelet lysates (~3 nM) (Fig. 3). At saturation, 3.66 fmol [^{3}H]dihydroergocryptine were bound/10^7 platelets. This corresponds to 220±45 receptor sites/platelet (n = 13). Thus, the numbers of binding sites determined from experiments with platelet lysates and intact platelets are in excellent agreement (220±26 vs. 220±45).

The specificity of [^{3}H]dihydroergocryptine binding to the intact platelets was also in good agreement with that determined in the lysates. Fig. 6 shows displace-

TABLE I

Inhibition of [^{3}H]Dihydroergocryptine Binding to Platelet Lysates by Adrenergic and Other Drugs

Drug	Inhibition of [^{3}H]DHE binding—K_d
	μM
Adrenergic	
Agonists	
Clonidine	0.017
(−)Epinephrine	0.26
(+)Epinephrine	2.3
(−)Norepinephrine	0.85
(+)Norepinephrine	17.0
(−)Phenylephrine	0.86
(−)Isoproterenol	142.0
Antagonists	
Yohimbine	0.002
Dibozane	0.004
Dihydroergocryptine	0.011
Phentolamine	0.014
Ergotamine	0.014
Dihydroergotamine	0.023
Ergocryptine	0.057
(+)Butaclamol	0.1
Haloperidol	2.6
Propranolol	12.0
Dichlorisoproterenol	15.7
Practolol	140.0
Others	
Dopamine	3.1
Serotonin	24.0
Dopa	N.I.
Catechol	N.I.
Normetanephrine	N.I.
Dihydroxymandelic acid	N.I.

Dissociation constants were calculated from the concentrations of the agents which caused 50% inhibition of [^{3}H]dihydroergocryptine binding using the relationship $K_d = EC_{50}/1 + (s/K_m)$ (23), where $[s]$ = the concentration of [^{3}H]dihydroergocryptine used in the binding assays (5.5 nM), and K_m = the dissociation constant of [^{3}H]dihydroergocryptine (2 nM). Each drug was tested two to eight times, and the mean value was recorded. NI indicates no inhibition at a concentration of 1 mM.

ment curves for phentolamine, (−) and (+)epinephrine. The displacement curves for phentolamine and (−)epinephrine in intact platelets are virtually superimposable on those obtained in platelet lysates although that for (+)epinephrine is displaced somewhat to the right.

Specificity of adenylate cyclase inhibition in platelet lysate. α-Adrenergic catecholamines such as epinephrine inhibit PGE_1-stimulated adenylate cyclase activity in platelets (10, 11). The pattern of inhibition by catecholamines appears to be that of an α-adrenergic response. Accordingly, we utilized epinephrine-stimulated inhibition of PGE_1-stimulated adenylate cyclase

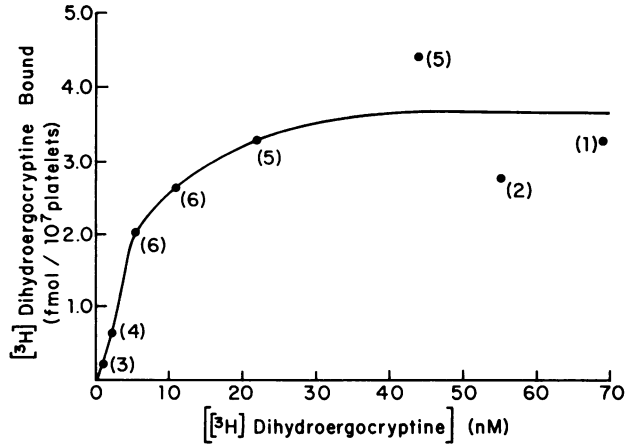

FIGURE 5 Specific binding of [^{3}H]dihydroergocryptine to intact human platelets as a function of [^{3}H]dihydroergocryptine concentration. Intact platelets were incubated with the indicated concentrations of [^{3}H]dihydroergocryptine, and binding was determined as described under Methods. Each value shown is the mean of duplicate determinations from the number of experiments shown in parenthesis. Comparable data were obtained when platelets were incubated at 37°C.

in the platelet lysates as a convenient biochemical assay of α-adrenergic effects which could be used for correlation with the [^{3}H]dihydroergocryptine binding data.

α-Adrenergic catecholamine agonists inhibited enzyme activity with a relative potency order identical to that observed for inhibition of [^{3}H]dihydroergocryptine binding and typical of an α-adrenergic receptor mediated process, i.e., (−)epinephrine > (−)norepinephrine > (−)isoproterenol (Fig. 7). Inhibition of

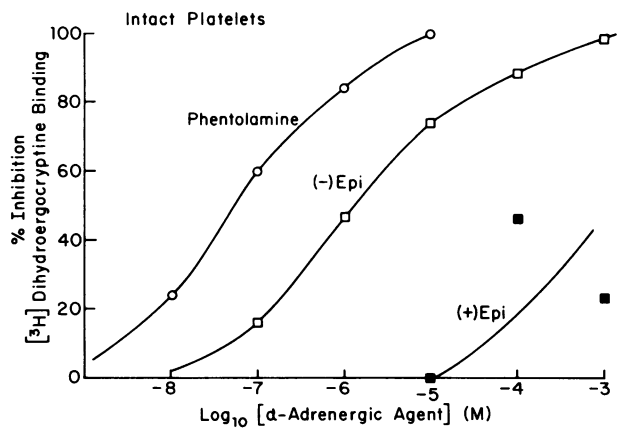

FIGURE 6 Inhibition of [^{3}H]dihydroergocryptine binding to intact platelets by adrenergic agents. Intact platelets were incubated with [^{3}H]dihydroergocryptine (~5 nM) in the absence and presence of the indicated agents, and specific binding was determined. Each value is the mean of duplicate determinations from five to six experiments.

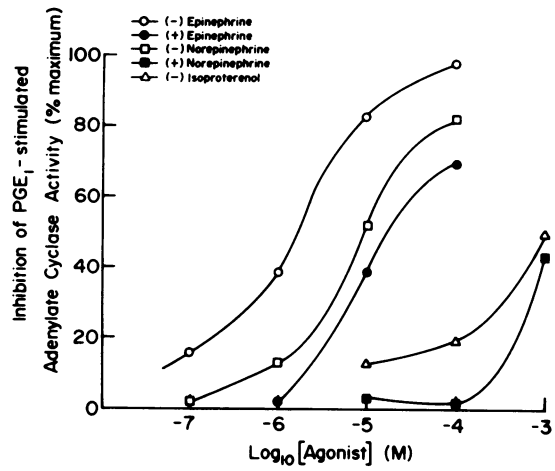

FIGURE 7 Inhibition of PGE_1-stimulated adenylate cyclase activity in human platelet lysates by adrenergic agonists. Maximum response refers to the decrease in cAMP generation produced by 1 mM (−)epinephrine. This decrease was generally a 40–60% reduction in PGE_1-stimulated enzyme activity. Values shown are the means of duplicate determinations from at least two separate experiments.

PGE_1-stimulated adenylate cyclase activity by these catecholamines also displayed stereospecificity comparable to that observed in the binding studies (Fig. 7).

Potent α-adrenergic antagonists such as phentolamine and dihydroergocryptine itself competitively antagonized the epinephrine-induced inhibition of enzyme activity (Fig. 8). The competitive nature of this antagonism is indicated by the parallel shifts in the epinephrine dose-response curve caused by these agents with the same maximum response achieved. From data such as that shown in Fig. 8, apparent dissociation constants could be calculated for phentolamine and dihydroergocryptine as antagonists of the α-receptor-mediated enzyme inhibition (see figure legend). These values were, for phentolamine 0.2 μM (n = 6), and for dihydroergocryptine 0.02 μM (n = 8). These values were somewhat higher than those calculated from binding studies. Dihydroergocryptine had no agonist effect of its own at concentrations up to 10 μM. Yohimbine was also a potent antagonist (K_d = 0.06 μM). In contrast, the classical β-adrenergic antagonist propranolol was very weak in antagonizing the epinephrine effect with a calculated K_d from adenylate cyclase studies of 89 μM.

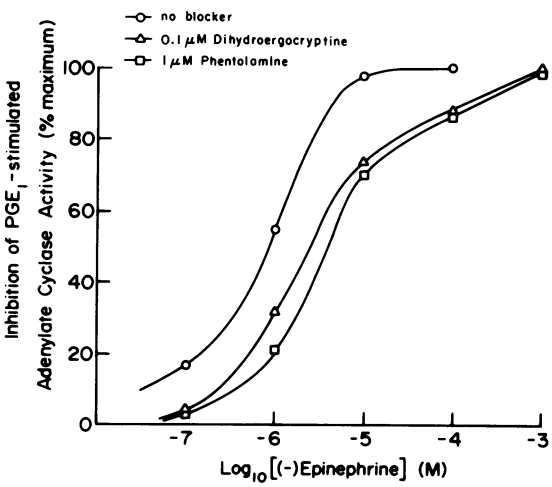

FIGURE 8 Antagonism of epinephrine induced inhibition of PGE_1-stimulated adenylate cyclase by phentolamine and dihydroergocryptine. Enzyme assays were performed in the presence of the indicated concentrations of epinephrine with or without the concurrent presence of phentolamine or dihydroergocryptine. Maximum response refers to the decrease in cAMP generation produced by 1 mM (−)epinephrine. These data were used to calculate K_ds of the antagonists using the relationship K_d = [antagonist]/(CR-1), where CR (concentration ratio) refers to the ratio of equiactive concentrations of (−)epinephrine in the presence and absence of the stated concentration of antagonist (24).

Phenylephrine appeared to be a "partial agonist". The inhibition of enzyme activity by phenylephrine ranged from 20–60% (n = 6) of that caused by epinephrine at the highest concentration studied (1 mM). Phenylephrine also antagonized the reduction in enzyme activity produced by epinephrine as would be expected of a partial agonist.

At 1 mM dopamine and serotonin caused slight inhibition of PGE_1-stimulated adenylate cyclase amounting to 40 and 16% of the maximal effect of epinephrine, respectively.

Effects of dihydroergocryptine on platelet aggregation. If the [^{3}H]dihydroergocryptine binding sites identified in the intact platelets and platelet lysates are in fact equivalent to the α-adrenergic receptors mediating the aggregating effects of epinephrine, then dihydroergocryptine binding should lead to antagonism of epinephrine-stimulated platelet aggregation. We found that dihydroergocryptine, 0.1 μM, inhibits the ability of epinephrine (30 μM) to aggregate platelets (data not shown).

DISCUSSION

[^{3}H]Dihydroergocryptine binding sites identified in human platelet lysates exhibit the specificity, stereospecificity, saturability, and kinetics to be expected of binding to physiological α-adrenergic receptors. The binding sites were also demonstrated in intact platelets. The binding characteristics agree closely with those of [^{3}H]dihydroergocryptine binding sites previously studied in smooth muscle membranes derived from rabbit uterus (14).

The ability of α-adrenergic agonists to inhibit PGE_1-sensitive adenylate cyclase in the platelet lysates directly paralleled their ability to inhibit [^{3}H]dihydro-

ergocryptine binding. Similarly, ability of adrenergic antagonists to antagonize enzyme inhibition and to inhibit [^{3}H]dihydroergocryptine binding were parallel. β-Antagonists were very weak both as inhibitors of [^{3}H]dihydroergocryptine binding and as antagonists of the epinephrine-induced inhibition of the adenylate cyclase. For example, propranolol was 450 times weaker than phentolamine as an antagonist in the enzyme assays and 850 times weaker in the binding assays. Although the relative potencies of the various adrenergic agents determined by [^{3}H]dihydroergocryptine binding and adenylate cyclase studies were comparable, somewhat higher concentrations of agents were necessary to produce effects on the adenylate cyclase than on [^{3}H]dihydroergocryptine binding. This is presumably due to a number of factors. (*a*) Differences in experimental conditions for the two types of assays: thus, for example, to optimize enzyme activity higher temperatures (37°C) were utilized, and PGE_1 was included in the enzyme assays; and (*b*) the presence of poorly understood "coupling factors" which affect the ultimate translation of receptor occupancy into biological response in the platelet lysates.

The activity of the PGE_1-stimulated adenylate cyclase which we observed in the platelet lysates was several-fold higher than that previously reported by several other laboratories (10, 19, 20). This may be due to differences in membrane preparation (freeze-thaw or sonication vs. mechanical disruption) or assay conditions (higher ATP and PGE_1 concentrations in our experiments).

The characteristics of [^{3}H]dihydroergocryptine binding to intact platelets appeared to be quite comparable to those observed in the platelet lysates. The number of receptors per platelet determined from the studies with lysates and intact platelets was also identical (220 per platelet). The ability of dihydroergocryptine to inhibit epinephrine-induced platelet aggregation lends further credence to the contention that the sites labeled are in fact equivalent to the physiological α-adrenergic receptors.

The specificity of the [^{3}H]dihydroergocryptine binding sites which we have identified appear to be identical to the previously demonstrated specificity of: (*a*) epinephrine-induced aggregation of platelets, which is blocked by phentolamine but not by β-antagonists (2–4); (*b*) epinephrine-induced reduction of PGE_1-stimulated cAMP levels in intact platelets (5–9); and (*c*) epinephrine-induced inhibition of PGE_1-stimulated adenylate cyclase in platelet lysates (10).

All of these phenomena have typical characteristics of an α-adrenergic receptor-mediated process. Nonetheless, it should be noted that both our data and previous reports (10, 21) suggest that the platelet α-adrenergic receptors may differ in certain respects from other peripheral α-adrenergic receptors. For example, phenylephrine is a classical full agonist at peripheral α-adrenergic receptors. However, it appeared to have only partial agonist activity in our study of adenylate cyclase and in previous reports was a partial agonist for reduction of cAMP levels in intact platelets (6). Phenylephrine also appears to be devoid of agonist activity in aggregating platelets (21).

The ability to study other hormone and drug receptors in circulating cells has provided a useful approach to the investigation of receptor abnormalities in human disease states (12, 22). The ability to quantitate and characterize [^{3}H]dihydroergocryptine binding sites in human platelets now extends this approach to the direct study of α-adrenergic receptors in normal and pathophysiologic states in man.

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