Identification of β-Adrenergic Receptors in Human Lymphocytes by (—)[^{3}H]Alprenolol Binding

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ABSTRACT Human lymphocytes are known to possess a catecholamine-responsive adenylate cyclase which has typical β-adrenergic specificity. To identify directly and to quantitate these β-adrenergic receptors in human lymphocytes, (—)[^{3}H]alprenolol, a potent β-adrenergic antagonist, was used to label binding sites in homogenates of human mononuclear leukocytes. Binding of (—)[^{3}H]alprenolol to these sites demonstrated the kinetics, affinity, and stereospecificity expected of binding to adenylate cyclase-coupled β-adrenergic receptors. Binding was rapid ($t_{\frac{1}{2}} < 30$ s) and rapidly reversible ($t_{\frac{1}{2}} < 3$ min) at 37°C. Binding was a saturable process with 75±12 fmol (—)[^{3}H]alprenolol bound/mg protein (mean±SEM) at saturation, corresponding to about 2,000 sites/cell. Half-maximal saturation occurred at 10 nM (—)[^{3}H]alprenolol, which provides an estimate of the dissociation constant of (—)[^{3}H]alprenolol for the β-adrenergic receptor.

The β-adrenergic antagonist, (—)propranolol, potently competed for the binding sites, causing half-maximal inhibition of binding at 9 nM. β-Adrenergic agonists also competed for the binding sites. The order of potency was (—)isoproterenol > (—)epinephrine > (—)-norepinephrine which agreed with the order of potency of these agents in stimulating leukocyte adenylate cyclase. Dissociation constants computed from binding experiments were virtually identical to those obtained from adenylate cyclase activation studies. Marked stereospecificity was observed for both binding and activation of adenylate cyclase. (—)Stereoisomers of β-adrenergic agonists and antagonists were 9- to 300-fold more potent than their corresponding (+)stereoisomers. Structurally related compounds devoid of β-adrenergic activity such as dopamine, dihydroxymandelic acid, normetanephrine, pyrocatechol, and phentolamine did not effectively compete for the binding sites. (—)[^{3}H]alprenolol binding to human mononuclear leukocyte preparations was almost entirely accounted for by binding to small lymphocytes, the predominant cell type in the preparations. No binding was detectable to human erythrocytes.

These results demonstrate the feasibility of using direct binding methods to study β-adrenergic receptors in a human tissue. They also provide an experimental approach to the study of states of altered sensitivity to catecholamines at the receptor level in man.

INTRODUCTION

Specific membrane receptor sites for a number of hormones have been identified in a variety of tissues by direct binding studies using biologically active radiolabeled compounds. The application of these techniques to the investigation of possible molecular alterations of hormone receptors in human disease has been limited by the relative inaccessability of large quantities of human tissue for binding studies. Recently, Soll et al. (1) demonstrated by direct binding studies that insulin receptor defects in thymic lymphocytes mirrored defects in insulin receptors in adipose and hepatic tissue from insulin-resistant animals. Subsequently, Archer et al. (2) used insulin binding studies in human peripheral leukocytes to detect insulin receptor defects in human insulin-resistant states.

Alterations in the β-adrenergic receptor have been proposed in human pathological states characterized by

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altered sensitivity to catecholamines. These include hyperthyroidism (3) and asthma (4). The identification of β-adrenergic receptors in a human tissue by direct binding studies would make possible the investigation of the role of β-adrenergic receptor alterations in these diseases. The presence of β-adrenergic receptors in circulating leukocytes has been indicated by the demonstration in these cells of adenylate cyclase activity that responds to catecholamines with a typical β-adrenergic specificity (5). However, these receptors have not previously been studied by direct binding methods.

Early attempts at identifying β-adrenergic receptors by direct binding methods with [^{3}H]catecholamines were associated with discrepancies between binding data and data from physiological response measurements. Thus, more refined and stringent criteria have been proposed (6, 7) for β-adrenergic receptor identification: (*a*) β-Adrenergic receptor binding should be rapid and reversible. (*b*) Receptor binding should be saturable indicating a finite number of binding sites. (*c*) β-Adrenergic agonists and antagonists should compete for the binding sites in a manner that closely parallels the known specificity of physiological β-adrenergic responses. Structurally related compounds which are devoid of physiological β-adrenergic effects should not compete for the binding sites. (*d*) (−)Stereoisomers of adrenergic agonists and antagonists should be considerably more potent than the (+)stereoisomers in competing for the binding sites, thus reflecting the stereospecificity observed in physiological β-adrenergic responses.

Recently we have used (−)[^{3}H]alprenolol, a potent competitive β-adrenergic antagonist, to identify binding sites in frog erythrocytes (8, 9) and canine myocardium (10) which satisfy all of these criteria. Here we report the first successful application of these direct binding techniques to the identification of β-adrenergic receptors in a human tissue, the circulating lymphocyte.

METHODS

Pharmacological agents

(−)[^{3}H]Alprenolol[1] used for these studies has a specific activity of 17 Ci/mmol and has biological activity and chromatographic properties identical to native (−)alprenolol (9). Other compounds used in this study were: (−)isoproterenol bitartrate, (−)epinephrine bitartrate, (−)norepinephrine bitartrate, (±)normetanephrine, dihydroxymandelic acid, and dopamine (Sigma Chemical Co., St. Louis, Mo.); (+)isoproterenol bitartrate, (+)epinephrine bitartrate, and (+)norepinephrine bitartrate (Winthrop Laboratories, N. Y.); (−)- and (+)propranolol hydrochloride (Ayerst Laboratories, N. Y.); phentolamine mesylate (Ciba Pharmaceutical Co., Summit, N. J.), and pyrocatechol (Mann Research Labs, N. Y.).

Leukocyte preparations

The various cell preparations were isolated from heparinized peripheral blood (100–200 cc) of healthy human volunteers as described below:

Mononuclear leukocytes (MNL's).[2] Blood was centrifuged on Ficoll-Hypaque density gradients using the method of Boyum (11). The MNL fraction contained approximately 85% small lymphocytes, 13% monocytes, and less than 2% polymorphonuclear leukocytes by morphological criteria using Türk's solution. These cells were removed from the gradients and washed in 0.02 M phosphate-buffered saline, pH 7.2 containing 0.1% gelatin and 5 mM $MgCl_2$ and 0.15 mM $CaCl_2$ (PBS). After centrifugation at 400 *g* for 15 min at 4°C, the mononuclear cell pellets were resuspended in 20 ml 50 mM Tris-HCl (pH 8.1 at 4°C) containing 10 mM $MgCl_2$ (incubation buffer) and homogenized in a Potter-Elvejhem glass homogenizer fitted with a motor-driven Teflon pestle. The homogenate was centrifuged at 25,000 *g* for 15 min, the pellet was washed twice with cold incubation buffer, and the final pellet was then resuspended in 3 ml of incubation buffer resulting in a suspension containing about 3 mg protein/ml. This mononuclear cell homogenate was used in all experiments unless otherwise specified.

Purified lymphocytes. Portions of the MNL fractions isolated from the Ficoll-Hypaque gradients were washed in PBS, standardized to contain 1×10^7 cells/ml and passed twice over a nylon mesh column at 37°C at a rate of 1 ml/min. This procedure, designed to remove monocytes and polymorphonuclear leukocytes which adhere to the nylon mesh, resulted in a purified lymphocyte preparation containing at least 99% small lymphocytes by morphological criteria. After one wash in PBS the cells were homogenized in incubation buffer, centrifuged, washed, and resuspended as described for the MNL preparation above.

Polymorphonuclear leukocyte (PMN) preparation. PMN preparations were obtained from the erythrocyte-PMN pellets produced after fractioning blood on Ficoll-Hypaque gradients. These pellets were diluted 1:2 with PBS and then mixed with an equal volume of 3% (wt/vol) high molecular weight dextran (T250, Pharmacia Fine Chemicals Inc., Piscataway, N. J.) in PBS. The erythrocytes were allowed to sediment for 25 min at 25°C and the supernate was removed. These supernates, containing at least 98% PMNs, were washed in PBS and then homogenized in incubation buffer, centrifuged, washed, and resuspended as described above for the MNL preparation.

Erythrocyte preparation. The erythrocyte sediments obtained after dextran sedimentation of the erythrocyte-PMN pellets derived from Ficoll-Hypaque gradients were washed three times in PBS. Erythrocytes were then homogenized in incubation buffer, centrifuged, washed, and resuspended as described above for the MNL preparation.

[1] "(−)[^{3}H]Alprenolol" refers to the compound which was prepared by New England Nuclear by catalytic reduction of (−)alprenolol (Hassle) with tritium gas using paladium as the catalyst. Mass spectroscopy of (−)[^{3}H]alprenolol has recently demonstrated that its structure is that of dihydroalprenolol. Hence tritiation of alprenolol probably occurs at the unsaturated bond in the aliphatic chain on position 2 of the aromatic ring.

[2] *Abbreviations used in this paper:* K_D, dissociation constant; MNL, mononuclear leukocyte; PBS, phosphate-buffered saline, pH 7.2 containing 0.1% gelatin and 5 mM $MgCl_2$ and 0.15 mM $CaCl_2$; PMN, polymorphonuclear leukocyte.

(−)[^{3}H]Alprenolol binding assay

Binding experiments were performed by incubating 250–350 μg leukocyte protein with (−)[^{3}H]alprenolol, (10 nM, unless otherwise specified), in a total volume of 150 μl of incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4 at 37°C) for 12 min at 37°C. This amount of leukocyte protein generally resulted in a receptor site concentration of 0.16 nM in the assay. In competition experiments, varying concentrations of agonists or antagonists were added to the incubation as indicated. Incubations were terminated by rapidly diluting a 125-μl incubation aliquot with 2 ml of ice-cold incubation buffer followed by rapid vacuum filtration of the diluted incubate through Whatman GFC glass fiber filters. The filters were rapidly washed by vacuum filtration of 10 ml of ice-cold incubation buffer. After drying, filters were placed directly into triton/toluene-based scintillation cocktail and counted in a Packard liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.) at an efficiency of 40%.

In each experiment "nonspecific" binding to homogenate protein was determined by measuring the amount of radioactivity retained on filters when incubations were performed in the presence of a high concentration (10 μM) of (±)-propranolol. "Specific binding," defined as total binding to homogenate protein minus nonspecific binding, was 80–90% of the total (−)[^{3}H]alprenolol bound to homogenate protein. A very small amount of (−)[^{3}H]alprenolol (about 0.2% of the total tracer filtered) was also adsorbed to the glass fiber filters. This small "filter blank" was not affected by the presence of unlabeled β-adrenergic agonists and antagonists. The binding values reported in all figures and tables refer to specific binding as defined above.

Adenylate cyclase assay

Adenylate cyclase activity of mononuclear cell homogenates was assayed as previously described (12). Mononuclear cell homogenates rather than intact cells were used in these adenylate cyclase assays so that the results would be directly comparable to the binding studies which were also done on mononuclear cell homogenates. Incubations were performed in a volume of 50 μl which contained 30 mM Tris HCl (pH 7.4), 5 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 15 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 Salomon et al. (13).

Protein was determined by the method of Lowry et al. (14).

RESULTS

Characteristics of (−)[^{3}H]alprenolol binding. Specific binding of (−)[^{3}H]alprenolol to washed mononuclear cell homogenates at 37°C was rapid ($t_{\frac{1}{2}} < 30$ s). The rapidity of this reaction at 37°C precluded an accurate estimate of the forward rate constant by these techniques. Binding was rapidly and totally reversible by the addition of 10 μM (±)propranolol to an equilibrated mixture of (−)[^{3}H]alprenolol and mononuclear cell homogenate ($t_{\frac{1}{2}} < 3$ min). Cooling the homogenates to 1–4°C slowed dissociation rates such that no dissociation could be detected 45 min after addition of 10 μM (±)propranolol. This virtual absence of dissociation of (−)[^{3}H]alprenolol from homogenates in the cold permitted the assay of specific binding by the vacuum filtration method.

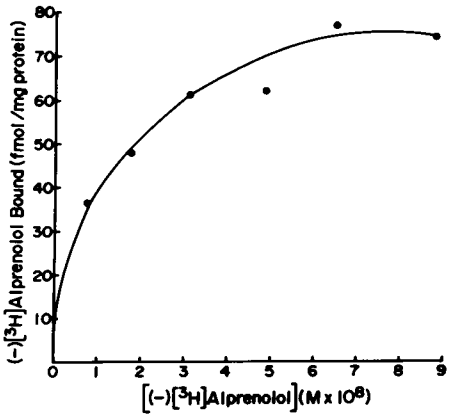

FIGURE 1 (−)[^{3}H]Alprenolol binding to mononuclear homogenates as a function of the concentration of (−)[^{3}H]-alprenolol in the incubation mixture. Specific binding was determined at various indicated concentrations of (−)[^{3}H]-alprenolol as described under Methods. Each value shown is the mean of determinations from duplicate incubations. The experiment shown is representative of three such experiments.

Binding was a saturable process with half-maximal saturation occurring at 10 nM (−)[^{3}H]alprenolol (Fig. 1). At saturation there were 75±12 fmol of (−)[^{3}H]-alprenolol bound/mg homogenate protein (mean±SEM, six experiments). Since $\sim 2 \times 10^7$ cells yielded 1 mg of homogenate protein, the saturation value corresponds to 2,000 sites per cell.

Specificity of (−)[^{3}H]alprenolol binding. β-Adrenergic agonists competed for the (−)[^{3}H]alprenolol binding sites (Fig. 2A) with an order of potency identical to the order of potency of these agonists in activating adenylate cyclase in human leukocytes (Fig. 2B): (−)isoproterenol > (−)epinephrine > (−)norepinephrine. Moreover, the absolute values of the dissociation constants computed from binding experiments were virtually identical with those derived from adenylate cyclase activation (Table I). The concentrations of the (+)stereoisomers of β-adrenergic agonists required to cause half-maximal inhibition of binding were 9–300-fold higher than those of the corresponding (−)stereoisomers, indicating marked stereospecificity of binding for the agonists. Similarly the adenylate cyclase response (Fig. 2B) to these agents was highly stereospecific.

(−)Propranolol, a β-adrenergic antagonist of isoproterenol-stimulated adenylate cyclase (Fig. 3), potently inhibited binding (Fig. 4) with half-maximal in-

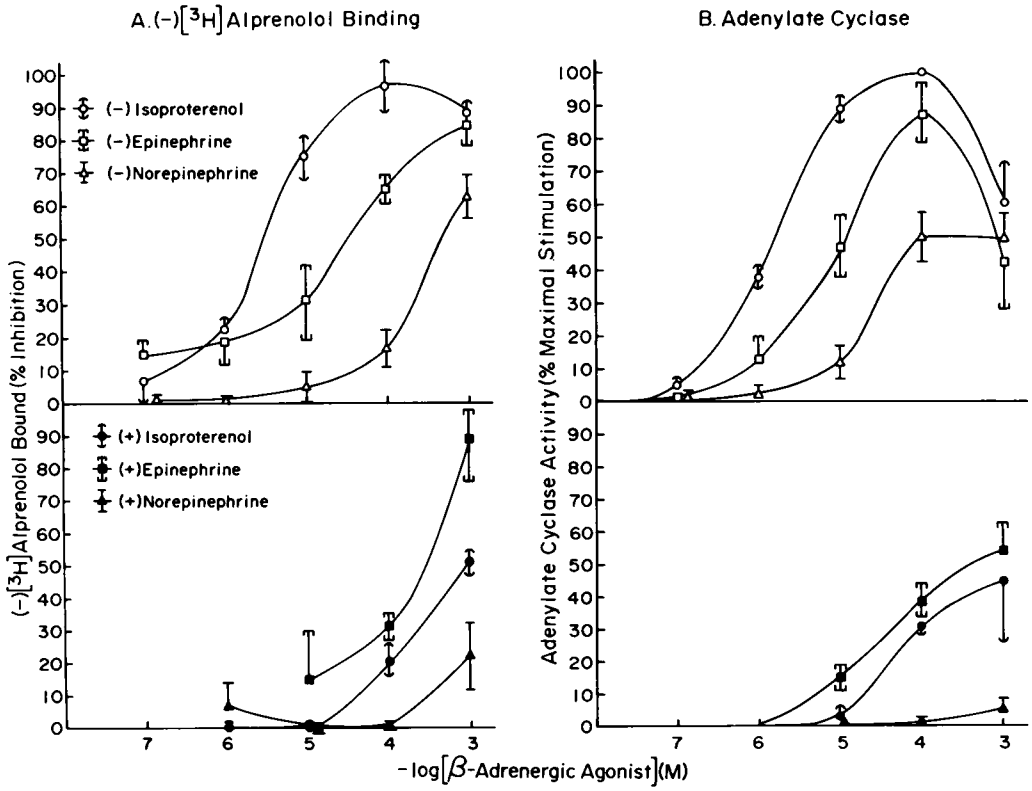

FIGURE 2 (A) Inhibition of (−)[^{3}H]alprenolol binding to mononuclear cell homogenates by the (−)stereoisomers and the corresponding (+)stereoisomers of isoproterenol, epinephrine, and norepinephrine. 100% inhibition refers to complete inhibition of specific binding. Each value shown is the mean±SEM of determinations from duplicate incubations from two to four separate experiments. (B) Stimulation of adenylate cyclase in mononuclear cell homogenates by adrenergic agonists. Adenylate cyclase assays were performed as described under Methods using the same mononuclear cell homogenate used in binding experiments. Adrenergic agonists were added from fresh stock solutions. Each point represents the mean±SEM of triplicate determinations from 2 to 10 separate experiments. Basal adenylate cyclase activity was 50 pmol/mg protein per min. Maximal stimulation was 30–45% above basal activity.

hibition occurring at 9 nM. This value can be used to calculate a K_D of 5 nM for the interaction of (−)propranolol with the (−)[^{3}H]alprenolol binding sites (Table I). This is in good agreement with the value of 8 nM computed for the K_D of propranolol as an antagonist of isoproterenol-stimulated adenylate cyclase (Fig. 3). Inhibition of (−)[^{3}H]alprenolol binding was highly stereospecific, the (−)stereoisomer of propranolol being 160 times more potent than the (+)stereoisomer.

(−)Alprenolol potently antagonized isoproterenol-stimulated adenylate cyclase (Fig. 3) with a calculated K_D of 9 nM. This value is in good agreement with the K_D for (−)[^{3}H] alprenolol (10 nM) estimated from the concentration of (−)[^{3}H]alprenolol which occupies 50% of the receptors (Fig. 1).

Compounds devoid of β-adrenergic physiological effects were not effective inhibitors of (−)[^{3}H]alprenolol binding. The catecholamine metabolites dihydroxymandelic acid and normetanephrine caused only 27 and 36% inhibition of binding at the high concentration of 1 mM. Likewise the catecholamine precursor, dopamine, and pyrocatechol at 1 mM inhibited only 35 and 15% of the binding, respectively. The potent α-adrenergic antagonist, phentolamine, caused only 30% inhibition of the binding at 0.1 mM, a concentration over four orders of magnitude higher than its physiological dissociation constant for the α-adrenergic receptor (15).

Cell type responsible for (−)[^{3}H]alprenolol binding. (−)[^{3}H]alprenolol binding to the mononuclear cell homogenate could be almost entirely accounted for by binding to the lymphocytic cells present in the preparations (Table I). Specific binding to purified lymphocyte preparations (99% lymphocytes, 1% monocytes) was not significantly different from binding to mononuclear cell preparations containing a 12-fold higher percentage of monocytes (86% lymphocytes, 12% monocytes). This

TABLE I

Apparent Dissociation Constants of β-Adrenergic Agents

Adrenergic agonist or antagonist	Dissociation constant (K_D)	
	(−)[^{3}H]Alprenolol binding	Adenylate cyclase
	μM	μM
(−)Propranolol	0.005	0.008
(−)Isoproterenol	1.2	0.9
(−)Epinephrine	11	11
(−)Norepinephrine	100	175

The concentrations of agonists required to half-maximally stimulate adenylate cyclase (Fig. 2B) were used as estimates of the dissociation constants (K_D) of the agonists as activators of adenylate cyclase. The K_D for propranolol as an antagonist of catecholamine-stimulated adenylate cyclase was calculated as in Fig. 3. Dissociation constants from binding experiments were calculated from the concentrations of β-adrenergic agonists required to inhibit 50% of the (−)[^{3}H]alprenolol binding (EC_{50}) by two methods. Using the equations of Cheng and Prusoff (19), the K_D for an agent which inhibits (−)[^{3}H]alprenolol binding is described by: $K_D = EC_{50}/(1 + [(-)[^3H]\text{alprenolol}]/K^*)$, where K* is the independently determined K_D for the interaction of (−)[^{3}H]alprenolol with its binding site (Fig. 1). Dissociation constants were also calculated by the method of Rodbard and Lewald (18) using the relationship: EC_{50}(adrenergic agent)/EC_{50}(alprenolol) = K_D(adrenergic agent)/K*, where EC_{50}(alprenolol) is the concentration of unlabeled (−)Alprenolol required for 50% inhibition of (−)[^{3}H]alprenolol binding and K* is defined as above. This treatment makes the valid assumption that the concentration of binding sites (0.16 nM) in the assay is small compared to the K_D of (−)[^{3}H]alprenolol (10 nM). The K_D's determined by these two methods agreed within 25% and the mean of the K_D's calculated by these methods is shown.

finding precludes the possibility that monocytes are responsible for a major portion of the (−)[^{3}H]alprenolol binding observed in mononuclear cell homogenates. Purified polymorphonuclear leukocytes demonstrated specific binding comparable to that observed in the mononuclear cell preparation (Table I). Since the PMN contamination of the mononuclear cell preparations is less than 2%, however, the binding due to contaminating PMNs in the preparation is insignificant. The absence of binding to human erythrocytes is in agreement with the previous failure to demonstrate catecholamine-sensitive adenylate cyclase activity in human erythrocytes (16).

DISCUSSION

The (−)[^{3}H]alprenolol binding sites studied in these mononuclear cell homogenates display the kinetics, saturability, stereospecificity, and affinity for adrenergic agonists and antagonists expected of true β-adrenergic receptors. The binding characteristics of these sites agree closely with those of (−)[^{3}H]alprenolol binding

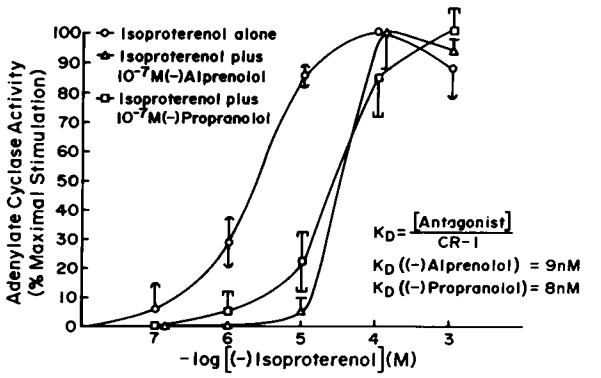

FIGURE 3 Inhibition of isoproterenol-stimulated adenylate cyclase by (−)alprenolol and (−)propranolol. Adenylate cyclase assays were performed as described in Methods. For curves done in the presence of antagonist, the antagonist was added to homogenates just before exposure to (−)isoproterenol in the adenylate cyclase assay. Each value shown is the mean±SD of three to six determinations. Basal adenylate cyclase activity was 50 pmol/mg protein per min. Maximal stimulation was 50% above basal activity. The K_D was calculated using the equation (Reference 15): K_D = [antagonist]/(CR-1) where CR (concentration ratio) is the ratio of equiactive concentrations of isoproterenol in the presence and absence of a given fixed concentration of the antagonist.

sites in amphibian erythrocytes (8, 9) and canine myocardium (10). The kinetics of (−)[^{3}H]alprenolol binding are consistent with the rapid forward and reverse rates found previously for catecholamine activation of adenylate cyclase-coupled β-adrenergic receptors (9). The number of (−)[^{3}H]alprenolol binding sites per lymphocyte (2,000) is similar to the reported values of 6,800 for the number of insulin receptors per thymic

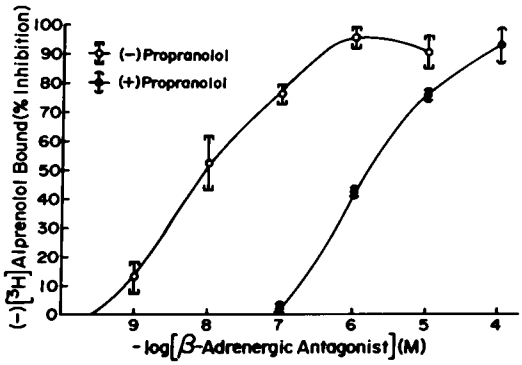

FIGURE 4 Inhibition of (−)[^{3}H]alprenolol binding to mononuclear cell homogenates by (−) and (+) stereoisomers of propranolol. (−)[^{3}H]alprenolol binding was determined in the presence of various concentrations of propranolol as described under Methods. 100% inhibition refers to complete inhibition of specific binding. Each value is the mean ±SEM of duplicate incubations from two or four separate experiments.

TABLE II

Specific Binding of (−)[³H]Alprenolol to Various Preparations of Human Circulating Cells

	L	M	PMN	Specific binding
	%	%	%	*fmol/mg protein*
Experiment I				
Leukocyte preparation				
Mononuclear cell	86	12	2	56
Purified lymphocytes	99	1	0	62
PMN leukocytes	2	0	98	77
Erythrocyte preparation				1
Experiment II				
Leukocyte preparation				
Mononuclear cells	86	13	1	57
Purified lymphocytes	99	1	0	50
PMN leukocytes	2	0	98	62
Erythrocyte preparation				0

Preparations for each experiment were obtained from a single donor as described under Methods and were incubated in binding assays at a concentration of 2–3 mg protein/ml with 20 nM (−)[³H]alprenolol. Cell counts were determined by counting at least 300 cells in a hemocytometer before homogenization. Leukocyte preparations contained 1–4 × 10^7 leukocytes/ml while erythrocyte preparations contained less than 5 × 10^4 leukocytes ml in a total volume of 20 ml. Values of specific binding represent means of determinations from triplicate incubations varying from the mean less than 7%. L, M, and PMN refer to lymphocytes, monocytes, and polymorphonuclear leukocytes identified by morphological criteria in Türk's solution.

lymphocyte (1), 4,000 for the number of growth hormone receptors per lymphocyte (17), and 1,800 for the number of β-adrenergic receptors per amphibian erythrocyte (9).

The equilibrium dissociation constants for the interaction of β-adrenergic agonists with the (−)[³H]alprenolol binding sites were calculated from the concentrations required to inhibit 50% of the specific (−)[³H]-alprenolol binding (Table I). These calculations, based on the method suggested by Rodbard and Lewald (18) and on the equation of Cheng and Prusoff (19) take into account the presence of the ligand (−)[³H]alprenolol at concentrations near its K_D in binding experiments designed to estimate the K_D of a competing unlabeled adrenergic agent. For each agonist there is excellent agreement between the K_D computed from binding data and the K_D derived from the concentration causing half-maximal stimulation of the enzyme adenylate cyclase. Furthermore, the K_D calculated from the inhibition of (−)[³H]alprenolol binding by the antagonist propranolol is comparable to the K_D estimated by the shift caused by propranolol in the curve of adenylate cyclase response to isoproterenol (Table I). For both adrenergic agonists and antagonists, there was marked stereospecificity in binding. Again, this was reflected in the response of the leukocyte adenylate cyclase to the stereoisomers of adrenergic agents (Fig. 2). Thus the specificity, affinity, and stereospecificity characteristic of β-adrenergic physiological responses were apparent in both the binding of (−)[³H]alprenolol and in the adenylate cyclase response in mononuclear cell homogenates.

A previous study of catecholamine-stimulated cyclic AMP accumulation in intact leukocytes by Bourne and Melmon (5) reported an order of potency (isoproterenol = epinephrine > norepinephrine) which is discrepant with the potency order (isoproterenol > epinephrine > norepinephrine) reported in the present study for catecholamine activation of adenylate cyclase in leukocytes. The order of potency reported by Bourne and Melmon is discrepant with not only the data in the present study but with the vast majority of data in the literature on β-adrenergic mediated processes in which isoproterenol is significantly more potent than epinephrine. The explanation for this discrepancy is not understood at this time.

It is of interest that the (−)[³H]alprenolol binding to the mononuclear cell homogenates can be accounted for almost entirely by binding to lymphocytes, the predominant cell type in the preparations. Erythrocyte contamination of the preparations does not affect binding since human erythrocytes do not contain adenylate cyclase-coupled β-adrenergic receptors (16) and do not bind (−)[³H]alprenolol (Table II). The small number of polymorphonuclear leukocytes contaminating the mononuclear cell preparations (2%) contributes negligibly to the total binding. Specific binding to the mononuclear cell preparations is not significantly altered by depletion of the monocyte population (Table II). Thus the amount of (−)[³H]alprenolol bound per milligram monocyte protein is less than or equal to that bound per milligram lymphocyte protein. Since the number of monocytes (12%) in a mononuclear cell preparation is small relative to the number of lymphocytes present (86%), most of the total binding is accounted for by binding to the lymphocytes. This is in marked contrast with the binding of ^{125}I-insulin to mononuclear leukocyte preparations which has been demonstrated to be mostly due to the monocytes present.

The existence of β-adrenergic receptors in human leukocytes has been indirectly demonstrated previously by adenylate cyclase activation studies (5) and by blast transformation studies (20) using β-adrenergic agonists and antagonists. Other authors have demonstrated the existence of β-adrenergic mediated responses in lymphoid tissue from rats and mice (21–23). Although the physiological functions of the β-adrenergic receptors in lymphocytes have not been fully elucidated, β-adrenergic receptor-mediated changes in cyclic adenosine 3′,5′-monophosphate levels are thought to play a role in modulating cytolytic activity of lymphocytes (22), antibody

production by lymphocytes (24), and lymphocyte maturation (25).

The direct identification of β-adrenergic receptors in these preparations from human circulating cells is significant in several respects. First, it demonstrates the feasibility of extending to human tissues binding methods originally developed in amphibian (8, 9) and avian (26, 27) erythrocytes. Second, the ability to characterize and quantitate β-adrenergic receptors in these preparations provides a method for the study of possible β-adrenergic receptor defects in human disease.

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