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CHARACTERIZATION OF (−)-[^{3}H]DIHYDROALPRENOLOL BINDING TO INTACT AND BROKEN CELL PREPARATIONS OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES †

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In this study we compared characteristics of (−)-[^{3}H]dihydroalprenolol ([^{3}H]DHA) binding sites in crude membrane preparations of human peripheral blood lymphocytes with those of intact, viable cells. A valid determination of specific β-adrenergic receptor binding in both preparations was obtained by defining non-specific [^{3}H]DHA binding with 10^{-6} M l- or dl-propranolol or 10^{-3} M l-isoproterenol. Higher concentrations of propranolol were used in prior reports on lymphocyte membranes. We showed that these concentrations may inhibit non-specific binding, causing non-saturability and inhomogeneity of the estimated 'specific' binding. In the intact cell preparations, inclusion of 10^{-4} M phentolamine was necessary to reduce the high degree of non-specific binding. By contrast, phentolamine (10^{-4} M) showed no effect on the [^{3}H]DHA binding to membrane preparations. At 37°C the [^{3}H]DHA binding to β-adrenergic receptor sites in both intact and broken cell preparations was rapid and reversible. The sites were stereoselective, as l-propranolol was about two orders of magnitude more potent to inhibit [^{3}H]DHA binding than was the d-isomer. In both preparations, agonists competed for specific binding with a rank order of potency isoproterenol > epinephrine > norepinephrine, which indicated a β_2-type of adrenergic receptor. The specific [^{3}H]DHA binding was saturable and Scatchard analysis revealed comparable numbers of homogeneous, non-cooperative binding sites (approximately 1250 receptors/cell in the membrane preparations and 1700 receptors/cell in the intact cells). In spite of these similarities the membrane sites showed a lower affinity for the antagonists [^{3}H]DHA and propranolol than did the intact cell sites, whereas their affinity for the agonists was increased. These differences indicate that the membrane system might be less suited to provide physiologically significant information about the β-adrenergic receptor system.

β-Adrenergic receptor [^{3}H]Dihydroalprenolol Human blood lymphocytes Lymphocyte membranes

1. Introduction

The direct identification of β-adrenergic receptors by (−)-[^{3}H]dihydroalprenolol ([^{3}H]DHA) binding is widely done with a variety of intact and broken cell preparations, including those of human peripheral blood lymphocytes (Williams et al., 1976; Krall et al., 1980). Since [^{3}H]DHA also binds to non-receptor sites in these preparations exact discrimination of specific- from non-specific binding is of great importance. Binding velocity, reversibility, saturability and (stereo-)specificity are generally accepted as the criteria for defining specific β-adrenergic receptor binding. Nevertheless, considerable differences have been reported in the characteristics of specific [^{3}H]DHA binding to human lymphocyte membrane preparations (e.g. Williams et al., 1976; Sheppard et al., 1977; Brooks

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et al., 1979; Bishopric et al., 1980; Davies and Lefkowitz, 1980; Tohmeh and Cryer, 1980). Although differences in cell disruption procedures may have contributed to this variability (Davies and Lefkowitz, 1980), another important factor may be the different ways which have been used to define non-specific binding. To this aim, the various authors have used a wide range (1–50 μM) of propranolol concentrations and mM concentrations of isoproterenol to inhibit specific binding. In most of these reports the representation of the [^{3}H]DHA binding characteristics is inadequate or largely ignored, making it difficult to evaluate the validity of the determinations. Moreover, in some cases the interpretation of binding data is made doubtful by considering non-saturable, low affinity binding as β-adrenergic receptor binding and/or by linearizing Scatchard plots with a curvilinear character. Therefore we considered it of prime importance to reinvestigate the characterization of specific- and non-specific [^{3}H]DHA binding to human lymphocyte membrane preparations in kinetic and equilibrium binding experiments, using the criteria mentioned above.

Although radioligand binding studies with broken cell preparations have proven to be invaluable in fundamental and clinical β-adrenergic receptor research (Williams and Lefkowitz, 1978), they do not permit a direct comparison of receptor binding with whole cell physiology. Moreover, evidence exists that cellular disruption and membrane purification may cause changes in β-adrenergic binding characteristics and adenylate cyclase activity (Insel and Stoolman, 1978; Terasaki and Brooker, 1978). Therefore, in this study, we compared [^{3}H]DHA binding characteristics of lymphocyte membrane preparations with those of intact cells. A suitable procedure for the determination of β-adrenergic receptors on intact, viable lymphocytes is described.

2. Materials and methods

2.1. Isolation of blood lymphocytes and preparation of membranes

Peripheral blood lymphocytes were isolated by density gradient centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) according to Bøyum (1968). The lymphocyte fraction was washed three times, either in Minimal Essential Medium (MEM), pH 7.4 (Gibco, Grand Island, N.Y., USA) supplemented with 10% decomplemented (56°C, 30 min) foetal calf serum (intact cell experiments), or in 25 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 0.6 mM $CaCl_2$, 5 mM glucose and 0.03% human albumin (membrane experiments). In both cases, cell viability was higher than 95% as assessed by trypan blue exclusion. The cell preparations contained approximately 90% lymphocytes, 10% monocytes and $< 2\%$ polymorphonuclear leukocytes.

Crude lymphocyte membranes were prepared by swelling intact cells in 50 mM Tris-HCl, 10 mM $MgCl_2$, pH 8.1 for 20 min at 0°C, followed by homogenization in an ice-cooled Potter-Elvehjem homogenizer (25 strokes). The membranes were centrifuged at $36\,000 \times g$ for 20 min (4°C) and washed once in 50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4. Finally the membranes were suspended in the same buffer at a concentration of about 2 mg protein/ml. Protein was measured according to Lowry et al. (1951). The mean protein yield was 1 mg/3.0 ($\pm$0.1) $\times 10^7$ cells.

2.2. [^{3}H]DHA binding assay

2.2.1. Lymphocyte membranes

In equilibrium studies, 100 μl aliquots of crude membranes (about 200 μg protein) were incubated in 50 mM Tris, 10 mM $MgCl_2$, pH 7.4 with several concentrations of [^{3}H]DHA in the absence or presence of various concentrations of unlabelled competing ligand, with or without phentolamine as indicated, for 15 min at 37°C (final vol 150 μl). In competition experiments, 1 mM ascorbic acid was included in the incubation medium in order to prevent oxidation of agonists. This agent does not affect specific binding as defined below. In kinetic studies the membranes were incubated with approximately 5 nM [^{3}H]DHA for various intervals of time as indicated. Incubations were stopped by the addition of 5 ml ice-cold 50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4 to the tubes and filtration over Whatman GF/C filters. Rinsing was per-

formed with 10 ml of the same buffer. Filters were counted in 10 ml Plasmasol (Packard, U.S.A.) in a liquid scintillation spectrometer. All results were corrected for counting efficiency (about 45%). Non-specific [^{3}H]DHA binding was defined as [^{3}H]DHA binding in the presence of 10^{-6} M l-propranolol (see results), and amounted to 15–55% of the total binding at [^{3}H]DHA concentrations of 0.5–20 nM. Specific binding was defined as the total binding minus the non-specific binding.

2.2.2. Intact lymphocytes

Equilibrium [^{3}H]DHA binding to intact cells was measured by incubating samples of 5×10^6 lymphocytes (suspended in MEM) with several concentrations of [^{3}H]DHA, in the absence or in the presence of various concentrations of competing unlabelled ligand, with or without phentolamine as indicated, for 15 min at 37°C (final vol of 325 μl). In kinetic studies the cells were incubated with 1.5–2 nM [^{3}H]DHA for various intervals of time as indicated. Incubations were stopped by the addition of 2 ml ice-cold MEM, followed by filtration under suction on Whatman GF/C glass fiber filters. The filters were washed rapidly with 15 ml ice-cold saline. After drying, the filters were counted in 9 ml of Insta-Gel (Packard, U.S.A.). Non-specific binding was determined in the presence of 10^{-6} M l-propranolol or 10^{-3} M l-isoproterenol. In the presence of 10^{-4} M phentolamine, non-specific binding was less than 50% at [^{3}H]DHA concentrations $\leqslant 2$ nM.

2.3. Materials

(−)-[^{3}H]Dihydroalprenolol (about 50 Ci/mmol) was purchased from New England Nuclear, U.S.A.; phentolamine was obtained from Ciba-Geigy, Switzerland, l- and d-propranolol were a kind gift from ICI, U.K. All other pharmacological agents were obtained from Sigma Chemical Company, U.S.A.

2.4. Statistics

Student's t-test for unpaired observations was used for statistical analyses. The level of significance was set at $P = 0.05$.

3. Results

3.1. Specificity of [^{3}H]DHA binding to lymphocyte membranes and intact lymphocytes

The most stringent criterion for discriminating between specific β-adrenergic receptor binding and non-specific binding of [^{3}H]DHA is the (stereo-)-

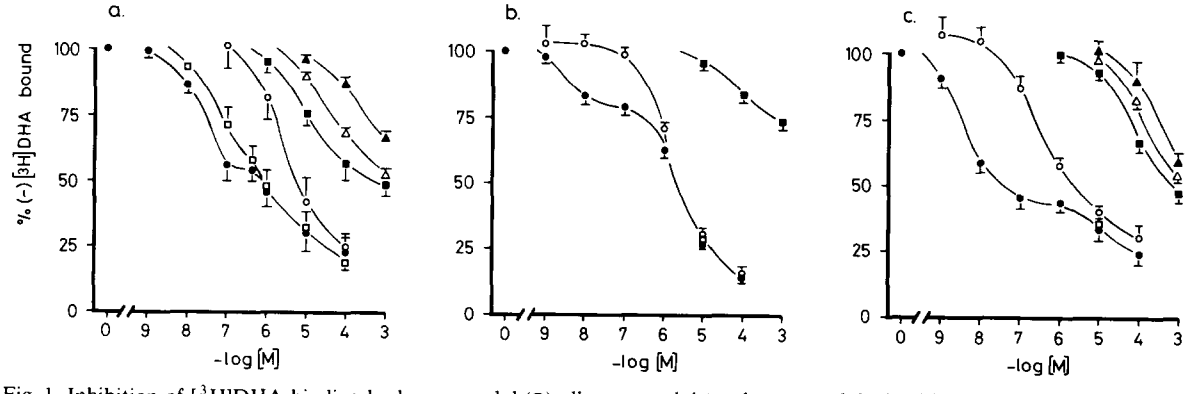

Fig. 1. Inhibition of [^{3}H]DHA binding by l-propranolol (●), dl-propranolol (□, d-propranolol (○), l-isoproterenol (■), l-epinephrine (△), and l-norepinephrine (▲). (a) Membrane preparations (5.5 nM [^{3}H]DHA; 100% binding represents 68.1 ± 9.2 (mean ± S.E.M.) fmol/mg protein. (b) Intact cells without phentolamine (1.6 nM [^{3}H]DHA; 100% binding represents 35.0 ± 5.0 fmol/5×10^6 cells), (c) Intact cells in the presence of 10^{-4} M phentolamine (1.6 nM [^{3}H]DHA; 100% binding represents 15.5 ± 4.0 fmol/5×10^6 cells). Each point is the mean ± S.E.M. of duplicate determinations from three to six separate experiments.

TABLE 1

Inhibition of [^{3}H]DHA binding to lymphocyte membranes and intact lymphocytes by β-adrenergic agonists and antagonists. The inhibition constants (K_i) for the competing agents were calculated from the equation (Cheng and Prussoff, 1973): $K_i = IC_{50}/1+(([^3H]DHA)/K_D)$. The IC_{50} values were derived from the mean inhibition curves shown in figs. 1a and 1c, and were defined as the concentrations of competing agent required to inhibit 50% of the specific [^{3}H]DHA binding. The K_D values for [^{3}H]DHA were independently estimated from the saturation studies shown in figs. 3a and 3b.

β-Adrenergic agent	K_i (μM)	
	Lymphocyte membranes	Intact lymphocytes
Antagonists		
l-propranolol	0.0076	0.0012
d-propranolol	0.59	0.095
Agonists		
l-isoproterenol	3.6	19.9
l-epinephrine	23.5	71.0
l-norepinephrine	93.8	143.4

selectivity of the labelled sites for agonists and antagonists, which can be assessed by competition experiments with the unlabelled ligands. Fig. 1 shows that in both the lymphocyte membranes (fig. 1a) and the intact lymphocytes (fig. 1b and 1c) [^{3}H]DHA binding was inhibited for at least two populations of binding sites. The agonists l-isoproterenol, l-epinephrine and l-norepinephrine inhibited [^{3}H]DHA binding to apparently one population of sites at concentrations up to 10^{-3} M, whereas the antagonists l-, dl- and d-propranolol at concentrations exceeding 10^{-6} M also competed for binding to an additional second population of sites. The first population of binding sites showed the binding characteristics of β-adrenergic receptors; first because l-propranolol was approximately 80 times more potent to inhibit [^{3}H]DHA binding than was d-propranolol, demonstrating the stereoselectivity of these sites (fig. 1a,b,c; table 1), and secondly because the agonist inhibition showed the potency order l-isoproterenol > l-epinephrine > l-norepinephrine (fig. 1a,c; table 1), which is indicative of adrenoceptors of the β_2-type. The second population of [^{3}H]DHA binding sites demonstrated a non-specific, non-β-adrenergic character, since agonists were unable to compete for these sites at the concentrations used while stereoselectivity for propranolol binding was lost. Accordingly, the inhibition curves of l-propranolol showed an intermediate plateau between concentrations of 10^{-7} and 10^{-6} M (fig. 1a,b,c), which allowed an accurate discrimination between specific and non-specific binding by this ligand. Therefore, 10^{-6} M l-propranolol was chosen for the estimation of non-specific binding in our system.

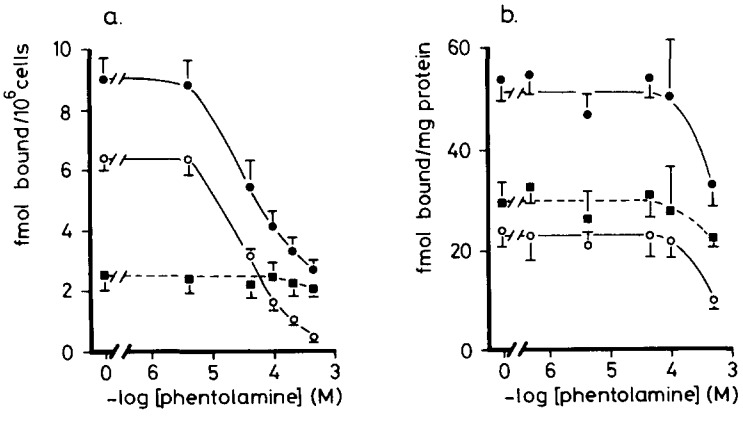

Fig. 2. The effect of different concentrations of phentolamine on total (●), non-specific (○), and specific (■) [^{3}H]DHA binding to: (a) intact cells (1.6 nM [^{3}H]DHA, non-specific binding determined with 10^{-3} M l-isoproterenol); (b) membranes (5.5 nM [^{3}H]DHA, non-specific binding determined with 10^{-6} M l-propranolol). Each point is the mean ± S.E.M. of duplicate determinations from three to four separate experiments.

When the K_i values for the inhibition of specific [^{3}H]DHA binding to membrane preparations were compared with those of intact cells differences appeared in receptor affinity between the two systems: the intact cell sites showed a 6-fold higher affinity for the antagonists than did the membrane sites, whereas their affinity for the agonists was several fold lower (table 1).

3.2. Effect of phentolamine on [^{3}H]DHA binding to intact lymphocytes and lymphocyte membranes

In contrast to the membrane preparations, intact lymphocytes showed a large degree of non-specific binding at low [^{3}H]DHA concentrations (fig. 1b), which complicated the exact determination of specific binding. This high non-specific binding could be considerably reduced by the addition of phentolamine to the incubation medium, without affecting the absolute amount of specific binding (fig. 2a), as was originally described by Sporn and Molinoff (1976) for [^{125}I]iodohydroxybenzylpindolol binding studies. At a concentration of 10^{-4} M phentolamine, the non-specific binding to the intact cells in the presence of 1.6 nM [^{3}H]DHA was decreased from approximately 75 to 45% of the total binding (figs. 1b,c and 2a). By contrast, up to 10^{-4} M phentolamine showed no effect on the membrane binding of [^{3}H]DHA (fig. 2b), which suggests that phentolamine acts mainly on the intracellular uptake of the ligand.

3.3. Saturability and affinity of [^{3}H]DHA binding to lymphocyte membranes and intact lymphocytes

At optimal binding conditions, using 10^{-6} M l-propranolol to determine non-specific binding, the specific [^{3}H]DHA binding to both lymphocyte membranes and intact lymphocytes was saturable and of high affinity (fig. 3a,b; table 2). The linear Scatchard plots revealed a homogeneous population of non-cooperative binding sites in both systems ($r = -0.96$ and $r = -0.99$, respectively; fig. 3a,b) and Scatchard analyses of the individual binding data demonstrated similar numbers of binding sites in both preparations (approximately 1250–1700 receptors/cell, table 2). However, the K_D value for specific [^{3}H]DHA binding was sig-

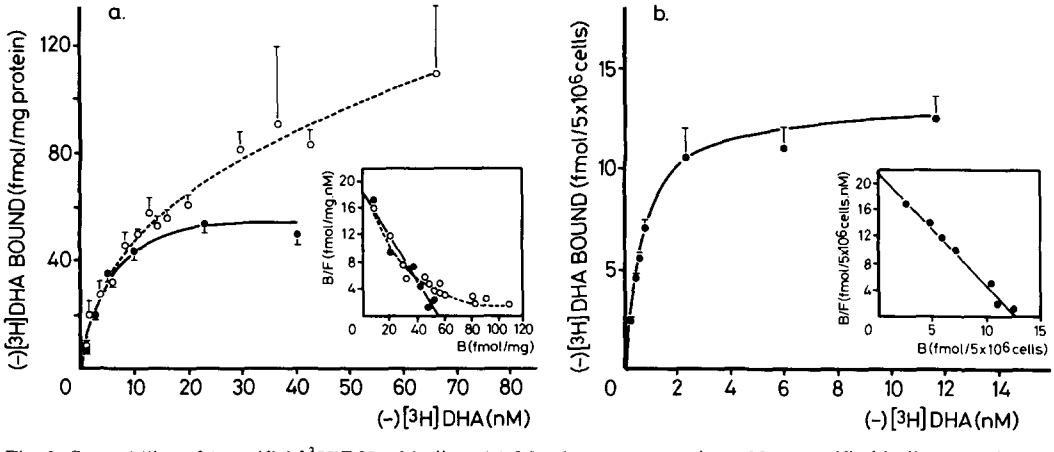

Fig. 3. Saturability of 'specific' [^{3}H]DHA binding. (a) Membrane preparations. Non-specific binding was determined with 10^{-6} M l-propranolol (●) and 10^{-5} M dl-propranolol (○). The inset shows the Scatchard analysis of the binding data. The Scatchard plot of the data obtained with 10^{-6} M l-propranolol indicates a K_D of 3.1 nM and a B_{max} of 56.5 fmol/mg protein, which corresponds to 1135 receptors/cell using the formula: receptors/cell = (mol of DHA bound)/(mg protein)×(mg protein yield)/(cell number)×(6.02×10^{23} molecules)/mol. The line is a linear regression line determined by least squares fit ($r = -0.96$). (b) Intact cells in the presence of 10^{-4} M phentolamine. Non-specific binding was determined with 10^{-6} M l-propranolol. The regression line in the inset ($r = -0.99$) indicates a K_D of 0.60 nM and a B_{max} of 13.0 fmol/5×10^6 cells, which corresponds to 1565 receptors/cell, using the formula: receptors/cell = (mol of DHA bound)/(cell number)×(6.02×10^{23} molecules)/mol. The binding data shown are means ± S.E.M. of duplicate determinations from two to six separate experiments.

TABLE 2

Parameters of [^{3}H]DHA binding to lymphocyte membrane preparations and intact lymphocytes. The numbers of binding sites (B_{max}) and apparent dissociation constants for [^{3}H]DHA (K_D) were derived from Scatchard analyses of five or six different saturation experiments. Each value is the mean ± S.E.M.

	Lymphocyte membranes (n = 6)	Intact lymphocytes (n = 5)	Significance
B_{max} (receptors/cell)	1262 ± 92	1724 ± 271	$P > 0.10$
K_D (nM)	4.2 ± 0.7	0.66 ± 0.14	$P < 0.005$

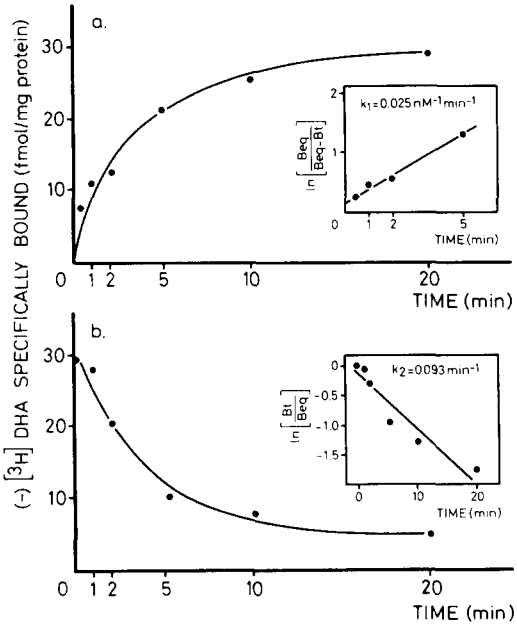

Fig. 4. Kinetic characteristics of the specific binding of [^{3}H]DHA (5.1 nM) to lymphocyte membrane preparations. (a) Time course for the association reaction. The inset shows a pseudo-first order kinetic plot of $\ln[B_{eq}/(B_{eq} - B_t)]$ against time, where B_{eq} is the amount of radioligand specifically bound at equilibrium and B_t represents the amount of radioligand specifically bound at time t. The line, determined by linear regression analysis ($r = 0.99$), has a slope of 0.222 min^{-1} which is equivalent to the observed initial rate constant (k_{ob}). The second order rate constant (k_1) for the association reaction was calculated from the equation $k_1 = (k_{ob} - k_2)/[[^3H]DHA]$, where k_2 is the rate constant for the dissociation reaction (see panel b). (b) Time course for the dissociation reaction. Lymphocyte membranes were incubated at 37°C for 20 min with [^{3}H]DHA (5.1 nM), followed by the addition of l-propranolol (10^{-6} M final concentration), which corresponds to t = 0. The specific binding of [^{3}H]DHA was determined at the indicated times and plotted as a function of time. The inset shows the first order kinetic plot of $\ln[B_t/B_{eq}]$ versus time. The negative slope of the line, determined by linear regression analysis ($r = -0.94$) is equivalent to the first order dissociation rate constant (k_2). The data shown are mean values from duplicate determinations and are representative of two similar experiments.

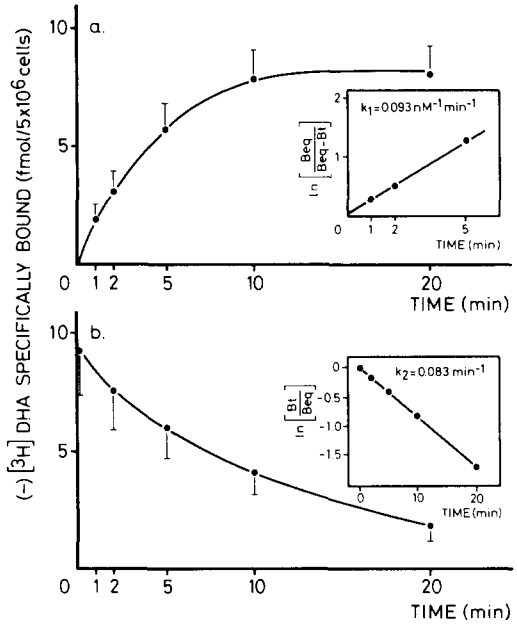

Fig. 5. Kinetic characteristics of the specific binding of [^{3}H]DHA (1.6 nM) to intact lymphocytes. (a) Time course for the association reaction. The inset shows a pseudo-first order kinetic plot of $\ln[B_{eq}/(B_{eq} - B_t)]$ against time, where B_{eq} is the amount of radioligand specifically bound at equilibrium and B_t represents the amount of radioligand specifically bound at time t. The line, determined by linear regression analysis ($r = 0.99$), has a slope of 0.260 min^{-1} which is equivalent to the observed initial rate constant (k_{ob}). The second order rate constant (k_1) for the association reaction was calculated from the equation $k_1 = (k_{ob} - k_2)/[[^3H]DHA]$, where k_2 is the rate constant for the dissociation reaction (see panel b). Each point is the mean value ± S.E.M. of five separate experiments performed in duplicate. (b) Time course for the dissociation reaction. Intact lymphocytes were incubated at 37°C for 15 min with [^{3}H]DHA (1.9 nM), followed by the addition of l-propranolol (10^{-6} M), which corresponds to t = 0. The specific binding of [^{3}H]DHA was determined at the indicated times and plotted as a function of time. The inset shows the first order kinetic plot of $\ln[B_t/B_{eq}]$ against time. The negative slope of the line, determined by linear regression analysis ($r = -0.99$) is equivalent to the first order dissociation rate constant (k_2). Each point is the mean ± S.E.M. of three separate experiments performed in duplicate.

nificantly lower for the intact cell system than for the membranes.

When the non-specific binding to the membrane preparations was determined with 10^{-5} M dl-propranolol instead of 10^{-6} M l-propranolol, as is often reported in literature, the estimated 'specific' binding appeared to be non-saturable and heterogeneous as indicated by the curvilinear Scatchard plot (fig. 3a). This indicated the presence of low affinity, non-receptor binding and thus confirmed the inhibition of non-specific binding by high concentrations of propranolol.

3.4. Kinetics of [^{3}H]DHA binding to lymphocyte membranes and intact lymphocytes

The specific binding of [^{3}H]DHA to both lymphocyte preparations was rapid ($t_{1/2}$ membranes = 3.1. min; $t_{1/2}$ intact lymphocytes = 2.7 min) and reversible ($t_{1/2}$ membranes = 7.4 min; $t_{1/2}$ intact lymphocytes = 8.3 min), (figs. 4a,b and 5a,b). At 37°C both association reactions reached equilibrium in 10–20 min (figs. 4a and 5a). The reaction rate constants k_1 and k_2 for both systems are presented in fig. 4 and fig. 5. From these rate constants, equilibrium dissociation constants ($K_D = k_2/k_1$) could be calculated which were in good agreement with those obtained from the saturation experiments. A K_D value of 3.7 nM was calculated for the membrane preparations from the kinetic experiments, versus a K_D of 4.2 nM from the equilibrium studies (table 2); in the intact cells these values were 0.89 and 0.66 nM respectively.

4. Discussion

In prior publications, considerable differences have been reported in the number and affinity of [^{3}H]DHA binding sites in broken cell preparations of human peripheral blood lymphocytes. Receptor densities in a range of 25 to over 1000 fmol/mg protein, or 500 to approximately 10.000 sites/cell were found, with K_D values ranging from 0.1 nM to 30 nM (table 3). Until now an extensive characterization of [^{3}H]DHA binding to human peripheral blood lymphocytes has only been described by Williams et al. (1976), who introduced the procedure with membrane preparations of these cells. They determined non-specific binding with 10^{-5} M dl-propranolol, a method which has also been applied by several other investigators. Al-

TABLE 3

Reported β-adrenergic receptor numbers and apparent dissociation constants of [^{3}H]DHA in binding studies with different definitions of non-specific binding.

Competing agent used for n.s. binding	B_{max}		K_D (nM)	References
	fmol/mg protein	receptors/cell		
(A) Lymphocyte membranes				
5×10^{-5} M dl-propranolol	~ 2000	N.r. [a]	N.r.	Brooks et al. (1979)
10^{-5} M dl-propranolol	75	2000	10	Williams et al. (1976)
10^{-5} M dl-propranolol	332–572	~ 8000–14000	20	Schocken and Roth (1977)
10^{-5} M dl-propranolol	682	~ 6000	30	Sheppard et al. (1977)
10^{-3} M isoproterenol/3×10^{-6} M propranolol	52	N.r.	1.5	Bishopric et al. (1980)
10^{-3} M l-isoproterenol	48	493	0.6	Davies and Lefkowitz (1980)
10^{-6} M l-propranolol	25	967	1.1	Ginsberg et al. (1981)
10^{-6} M l-propranolol	25–32	~ 1000	0.1–2.0	Tohmeh and Cryer (1980)
10^{-6} M l-propranolol	56	1262	4.2	This report
(B) Intact lymphocytes				
10^{-5} M dl-propranolol	–	2200	~ 1.0	Krall et al. (1980)
10^{-6} M l-propranolol	–	1725	0.7	This report

[a] Not reported.

though their experimental data seemed to justify the use of this concentration, we found that concentrations of propranolol exceeding 10^{-6} M may inhibit low affinity, non-receptor binding, in both membrane preparations and intact cells. This caused overestimation of the specific receptor binding, which was most pronounced at high [^{3}H]DHA concentrations, and which led to deviations from specific binding characteristics.

Inhibition of non-specific [^{3}H]DHA binding by high concentrations of propranolol has also been described for several other systems (Nahorski and Richardson, 1979; Mendel and Almon, 1979; Dulis and Wilson, 1980). Their experiments with membrane preparations of human erythrocytes and mouse brain led Mendel and Almon (1979) to suggest that non-specific [^{3}H]DHA binding to biological membranes is partially due to partitioning of the ligand into these membranes. The addition of propranolol, which also partitions into the membranes, may alter the membrane to a sufficient extent that the [^{3}H]DHA partition coefficient decreases, thereby causing inhibition of this binding. As in our study, they showed that the more hydrophilic agonists, isoproterenol and epinephrine, which have a much lower partition coefficient than the above mentioned antagonists had no significant influence on the non-specific [^{3}H]DHA association with the membranes.

Inhibition of non-specific binding might explain the aberrant binding characteristics and curvilinear Scatchard plots that can be observed or calculated from some earlier reports on lymphocyte preparations (Williams et al., 1976; Sheppard et al., 1977; Schocken and Roth, 1977; Brooks et al., 1979; Bishopric et al., 1980; Krall et al., 1980), and the related granulocyte system (Ruoho et al., 1980). Incorrect estimations of non-specific binding by high concentrations of competing ligand may thus have contributed to the differences in reported β-adrenergic binding characteristics. Our results indicate that the concentrations of competing ligand must be chosen carefully and that for this reason competition experiments with several competing ligands are necessary.

Under our conditions a valid determination of non-specific binding to both intact lymphocytes and lymphocyte membranes was obtained in the presence of 10^{-3} M l-isoproterenol or 10^{-6} M l- or dl-propranolol. Under these conditions the specific binding fulfilled the criteria proposed for β-adrenergic receptor identification. Agonists competed for the specific binding in a rank order of potency suggestive of β_2-adrenergic receptors. Stereoselectivity of the labelled sites was shown by the greater potency of the l-isomer than of the d-isomer of propranolol to inhibit binding. Additionally, receptor binding was saturable, rapid and reversible.

We showed that, next to membrane preparations, intact viable cells can be used to study β-adrenergic binding characteristics of human peripheral blood lymphocytes. The measurement of specific [^{3}H]DHA binding to the intact lymphocytes was complicated by a large extent of non-specific binding, which appears to be a common problem in intact cell systems (Terasaki and Brooker, 1978; Cabelli and Malbon, 1979; Pochet and Schmitt, 1979; Dulis and Wilson, 1980; Galant and Allred, 1981). The precise determination of specific binding was made feasible by the inclusion of phentolamine in the incubation medium. Several studies using intact cells have confirmed the usefulness of phentolamine in β-adrenergic receptor determinations (Cabelli and Malbon, 1979; Pochet and Schmitt, 1979; Pittman and Molinoff, 1980; Galant and Allred, 1981). A concentration of 10^{-4} M phentolamine considerably reduced the non-specific [^{3}H]DHA binding without affecting specific binding. The exact mechanism of phentolamine action is still not established. The absence of effect of this agent on the membrane preparations (fig. 2b) indicates that it acts mainly by inhibition of intracellular [^{3}H]DHA retention.

It has been proposed that the unprotonated permeable amine phentolamine may inhibit uptake of the radioligand into lysosomes by reducing the pH gradient between the medium and the acidic granules, analogous to the lysosomotropic amine chloroquine (Dulis and Wilson, 1980). This mechanism might also explain the competitive effect of propranolol, another permeable amine, on the high non-specific [^{3}H]DHA binding, whereas the more polar adrenergic ligand isoproterenol showed no significant inhibition of this binding (fig. 1b). A first study on intact lymphocytes was described

recently by Krall et al. (1980) who demonstrated 2200 receptors/ cell with a K_D for [^{3}H]DHA of about 1 nM. In contrast to our report, these authors did not present any evidence for the occurrence of high non-specific binding. However, it should be noticed that they used 10^{-5} M dl-propranolol to estimate non-specific binding, whereas we used 10^{-6} M l-propranolol. As already indicated, this difference might explain the apparent differences in specific and non-specific binding between the two studies, although the influence of other methodological differences (cell storage, medium, temperature) cannot be excluded in this case.

Under our conditions, the β-adrenergic receptor number measured on the intact lymphocytes (about 1700/cell) was not significantly different from that observed in the membrane preparations (approximately 1250/cell). Together with the similarities in binding kinetics and β_2-(stereo-)specificity of the labelled sites, these results indicate that identical β-adrenergic receptor sites were labelled in both lymphocyte preparations.

However, some differences were found in the affinity of the receptor for agonists and antagonists; β-adrenergic receptors in the membrane preparations had approximately a 6-fold lower affinity for the antagonists than did the receptors on the intact cells, whereas their affinity for the agonists was several times higher (tables 1 and 2). The latter discrepancy was also observed in preparations of S_{49} lymphoma cells (Insel and Stoolman, 1978), rat glioma cells (Terasaki and Brooker, 1978), human tonsil lymphocytes (Pochet et al., 1979) and L_6 muscle cells (Pittman and Molinoff, 1980). Insel and Stoolman (1978) suggested that the enhanced affinity for agonists of β-adrenergic receptors in membrane preparations of S_{49} lymphoma cells might be caused partially by a loss of endogenous GTP during cellular disruption and fractionation. They showed that in the absence of exogenously added guanyl nucleotides the potency of agonists to compete for binding sites in these membranes was one to two orders of magnitude greater than values observed in intact cells, whereas this difference was markedly reduced in the presence of these nucleotides. It is generally known that guanyl nucleotides are required for hormonal stimulation of the adenylate cyclase, and that they convert agonist binding sites from a high into a low affinity state (Ross et al., 1977; Kent et al., 1980). In addition, Pittman and Molinoff (1980) recently showed in L_6 muscle cells that an early stage of agonist-induced desensitization, which occurs in the intact cell system but not in the membrane preparations, may also play a role in the observed discrepancy.

Factors contributing to the decreased affinity of membrane receptors for the antagonists also remain to be elucidated. The difference with the intact cell system might be explained by a recent observation of Wolfe and Harden (1981), who showed an increased apparent affinity of the antagonists [^{125}I]iodohydroxybenzylpindolol, [^{125}I]-iodopindolol, and [^{125}I]iodocyanopindolol for the β-adrenergic receptors of L_6 myoblast membranes in the presence of exogenously added GTP, GDP and GppNHp. Thus, if cellular guanine nucleotides are washed out in our membrane preparations, this might lead to a decreased affinity of antagonists, in contrast to the increased affinity of the agonists. Other contributing factors might be the different ionic concentrations of the incubation media used for both systems and the possible changes in membrane structure induced by the cell disruption procedure. It should be noticed that other investigators using different homogenization procedures found K_D values comparable with those of the intact cell system (Davies and Lefkowitz, 1980; Ginsberg et al., 1981).

In conclusion, suitable experimental conditions were assessed to determine β-adrenergic binding sites on both lymphocyte membrane preparations and intact viable lymphocytes. The intact cell procedure provides the possibility for investigating the β-adrenergic receptor system under physiological conditions, retaining the integrity of the cell and all factors which are required for β-adrenergic receptor function. This includes in particular soluble factors such as guanine nucleotides and functional cytoskeletal elements, which may be lost in membrane preparations (Ross et al., 1977; Rudolph et al., 1977). A change in these factors may have caused the observed differences between β-adrenergic receptor affinity for agonists and antagonists in intact and broken cell preparations. These differences suggest that care must be taken

when extrapolating binding data obtained with membranes to intact cell physiology.

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