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Stereospecific Binding of Propranolol and Catecholamines to the β-Adrenergic Receptor

(stereospecificity/erythrocyte)

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ABSTRACT The stereospecificity of the β-adrenergic receptor of turkey erythrocyte ghosts was studied. Binding of the specific β-adrenergic antagonist, propranolol, was found to occur exclusively with the *l*-stereoisomer. The stereospecificity of catecholamine binding was determined by assaying the ability of the catecholamines to displace [3H]propranolol. Binding of the catecholamines was also found to be stereospecific for the *l*-stereoisomers. Furthermore, the *d*-stereoisomers do not compete with the *l* form for binding. Using this displacement technique, we were able to calculate the dissociation constants for *l*-epinephrine, *l*-norepinephrine, and *l*-isoproterenol from the β-receptor and compare these values to the ***apparent*** dissociation constants obtained from the direct activation of adenylate cyclase (EC 4.6.1.1) by these catecholamines.

Recently, we have demonstrated an experimental procedure for the quantitation of specific β-adrenergic receptors on a cell membrane containing an excess of nonreceptor catecholamine binding proteins (1). With this procedure, tritiated propranolol is used as the radioactive ligand rather than radioactive catecholamines. It was shown (1) that tritiated propranolol binds *exclusively* to the β-receptor and, thus, provides an extremely powerful tool for study of the binding properties of this receptor. Using this technique, we have measured the number of β-adrenergic receptors on the turkey erythrocyte cell. We found that each cell has about 1000 receptors. Furthermore, we have shown that [3H]propranolol binding is fully displaceable by excess isoproterenol and that it is stereospecific for the *l*-isomer of propranolol.‡ In this communication we report a full study of the stereospecificity of *catecholamine binding* to the β-receptor by measuring the capability of *l*-, *dl*-, and *d*-catecholamines to displace [3H]propranolol. The stereospecificity of the β-receptor for *l*-catecholamines‡ is demonstrated by direct binding studies. The strict stereospecificity for *l*-catecholamines for activation of adenylate cyclase has recently been demonstrated in a number of systems, including the turkey erythrocyte ghost (2, 3). It has also been demonstrated that the binding techniques using [3H]-catecholamines are inadequate for the characterization of the β-receptor since they measure largely, if not exclusively, binding to nonreceptor catecholamine binding proteins (2). Indeed, equilibrium dialysis studies of concentrated preparations of turkey erythrocyte ghosts using [3H]isoproterenol in the presence and in the absence of nonradioactive propranolol revealed that β-receptor sites constitute only a small fraction of the total catecholamine binding sites available on the membrane (2).

Abbreviation: $D_{0.5}$, concentration of displacing ligand that yields 50% displacement of [3H]propanolol.

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‡ These compounds possess the absolute configurations *R*. In our previous publication we used L and D where we should have used *l* and *d*, respectively.

EXPERIMENTAL

dl-[3H]Propranolol (4.3 Ci/mmole, over 99% pure) was obtained from the Israel Atomic Energy Commission (Negev). *l*-Isoproterenol, *dl*-isoproterenol, *l*-epinephrine, *dl*-epinephrine, *l*-norepinephrine, and *dl*-propranolol were obtained from Sigma. *d*-Epinephrine and *d*-propranolol were kindly donated by Prof. Z. Zelinger of the Hebrew University in Jerusalem. The different stereoisomers were both chromatographically and optically pure. Fresh turkey blood was obtained from a local slaughter house. Ghosts were prepared and assayed for adenylate cylcase [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)] activity as described elsewhere (1). Protein was determined according to the technique of Lowry *et al.* (4), with bovine serum albumin as a standard.

dl-[3H]Propranolol Binding was measured as described (1).

Displacement Experiments. In a typical displacement experiment, 10 nM *dl*-[3H]propranolol is incubated with a suspension of ghosts (1 mg/ml of protein) in the presence of increasing concentrations of the nonradioactive ligand. Incubations are in 50 mM Tris·HCl (pH 7.4) containing 0.5% (v/v) bovine serum albumin and 20 μg/ml of sodium metabisulfite. The final incubation volume is 1.0 ml, and the samples are incubated for 15 min at 25°. After incubation, the suspension is centrifuged at 40,000 rpm (100,000 × *g*) in a Spinco L-65 ultracentrifuge in the R-50 rotor for 15 min at 25°. The surface of the pellet and the sides of the tube are washed five times with 2.0 ml of 50 mM Tris·HCl (pH 7.4). Then the pellet is dissolved in a 1% sodium dodecyl sulfate solution in water. Samples are taken for determination of tritium and protein. Trapping is measured in each experiment by including 1 or 10 μM nonradioactive *dl*-propranolol. At 10 nM *dl*-[3H]propranolol, the trapping is between 56 and 60%. This value is subtracted from each value as described previously (1). Binding was found to be instantaneous (i.e., maximal binding occurs in less than 1 min).

Calculation of Dissociation Constants. At the saturated concentrations of [3H]propranolol used, the displacing ligand (L) competes with [3H]propranolol (PPL) for the receptor

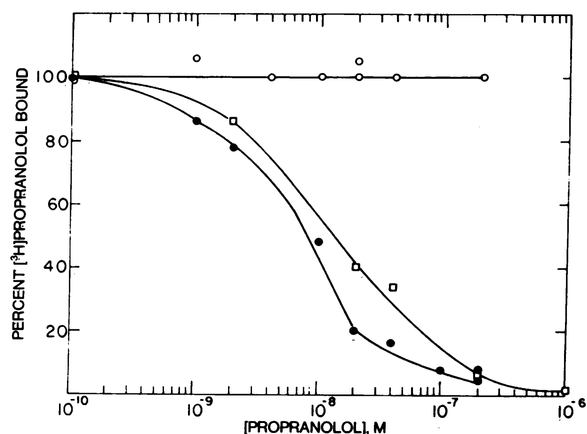

FIG. 1. The displacement of dl-[^{3}H]propranolol by nonradioactive propranolol. Experimental details are given in the *text*. Maximum specifically bound propranolol is consistently found to be 1.0–1.1 pmole/mg of ghost protein. Erythrocyte ghosts have an adenylate cyclase specific activity of 150–160 pmoles of cAMP formed per mg of protein per min. O, d-Propanolol; ●, l-propanolol, $D_{0.5} = 7 \times 10^{-9}$ M; □, dl-propanolol, $D_{0.5} = 1.3 \times 10^{-8}$ M.

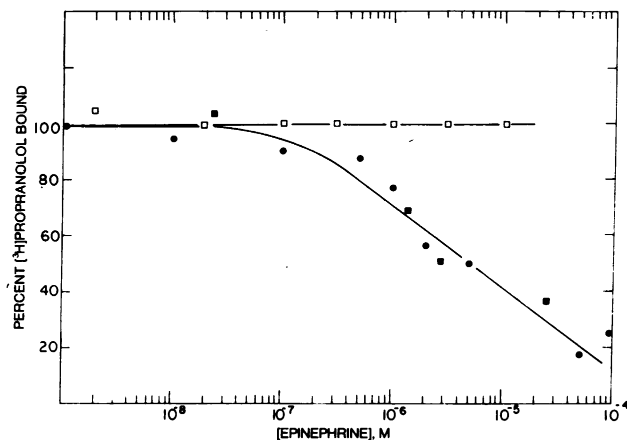

FIG. 2. The displacement of bound dl-[^{3}H]propranolol by epinephrine stereoisomers. ●, l-Epinephrine; ■, l-epinephrine + d-epinephrine, $D_{0.5} = 4.5 \times 10^{-6}$ M; □, d-epinephrine.

binding site (R):

$$\text{R-PPL} + \text{L} \rightleftarrows \text{RL} + \text{PPL} \qquad [1]$$

where

$$K = \frac{[\text{RL}] \times [\text{PPL}]}{[\text{R-PPL}] \times [\text{L}]} = \frac{K_{\text{PPL}}}{K_{\text{L}}} \qquad [2]$$

K_{PPL} is the dissociation constant for propranolol and K_{L} is the dissociation constant for the ligand. When 50% of the specifically bound [^{3}H]propranolol is displaced by L,

$$[\text{RL}] = [\text{R-PPL}]. \qquad [3]$$

Since

$$[\text{PPL}]_{\text{free}} \simeq [\text{PPL}]_{\text{total}} \qquad [4]$$

and

$$[\text{L}]_{\text{free}} \simeq [\text{L}]_{\text{total}} \qquad [5]$$

one obtains

$$K_{\text{L}} = K_{\text{PPL}} \frac{[\text{L}]_{\text{total}}}{[\text{PPL}]_{\text{total}}} \qquad [6]$$

One can, therefore, readily calculate the dissociation constant for the displacing ligand when the dissociation constant for propranolol is known (1). The dissociation constant for propranolol is obtained by a direct binding assay of [^{3}H]-propranolol, by the centrifugation technique as described (1).

We would like to propose the notation $D_{0.5}$ to specify the concentration of the displacing ligand that yields 50% displacement of [^{3}H]propranolol.

RESULTS

Propranolol Binding. The displacement of dl-[^{3}H]propranolol by the nonradioactive stereoisomers d- l-, and dl-propranolol is shown in Fig. 1. d-Propranolol is incapable of displacing the radioactive propranolol bound to the specific receptor. Furthermore, l-propranolol is about twice as efficient as dl-propranolol in its ability to displace [^{3}H]propranolol. This is reflected in the displacement curve (Fig. 1) and in the ligand concentration required for 50% displacement ($D_{0.5}$). The $D_{0.5}$ value for the dl racemic mixture is twice the $D_{0.5}$ value for the l-stereoisomer. This is in line with the finding that the d-isomer is incapable of displacing the bound [^{3}H]propranolol. The $D_{0.5}$ occurs at 6.5–7.0 nM with nonradioactive l-propranolol and at 13 nM with nonradioactive dl-propranolol. Since the total concentration of dl-[^{3}H]propranolol in the binding experiments is 11 nM, the experimental values obtained are close to the theoretical ones expected. The dissociation constant for l-propranolol can be computed from Eq. [6] and is indeed about half that measured for dl-propranolol by direct binding measurements (1).

Catecholamine Binding. The displacement of dl-[^{3}H]-propranolol by the stereoisomers of epinephrine, norepinephrine, and isoproterenol is shown in Figs. 2, 3, and 4. The dissociation constants for these catecholamines were computed from their $D_{0.5}$ values. Independently, the *apparent* dissociation constants for isoproterenol, epinephrine, and norepinephrine were obtained from the concentration dependence for adenylate cyclase activation (Table 1). d-Catecholamines do not stimulate adenylate cyclase (Table 1) and are incapable of displacing bound [^{3}H]propranolol (Figs. 2, 3, and 4).

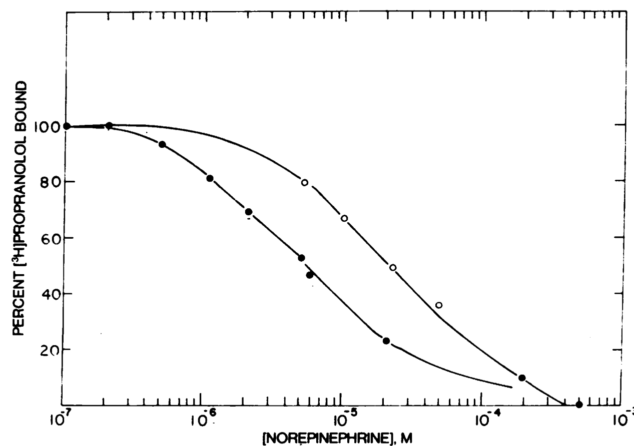

FIG. 3. The displacement of bound dl-[^{3}H]propranolol by norepinephrine stereoisomers. ●, l-Norepinephrine, $D_{0.5} = 6.0 \times 10^{-6}$ M; O, dl-norepinephrine, $D_{0.5} = 2.0 \times 10^{-6}$ M.

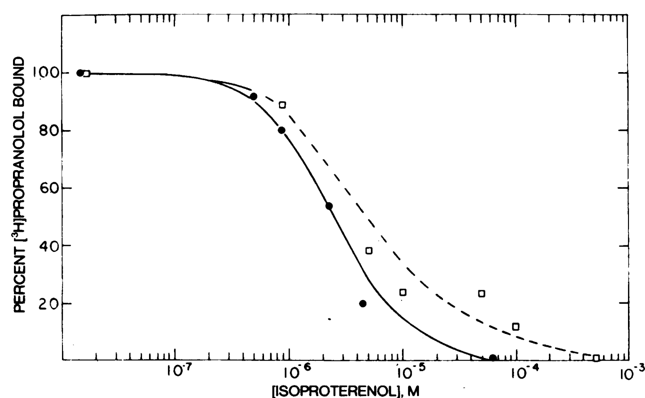

FIG. 4. The displacement of *dl*-[^{3}H]propranolol by isoproterenol stereoisomers. ●, *l*-Isoproterenol, $D_{0.5} = 2.6 \times 10^{-6}$ M; □, *dl*-isoproterenol, $D_{0.5} = 3.5 \times 10^{-6}$ M.

Furthermore, the racemic mixture *dl* is always less efficient than the *l*-isomer in displacing bound [^{3}H]propranolol (Figs. 2, 3 and 4). This is reflected in the finding that the $D_{0.5}$ value for the *dl* mixture is about twice the $D_{0.5}$ value for the *l*-stereoisomer, indicating that the *d*-stereoisomer does not displace [^{3}H]propranolol *and* is incapable of interfering with the displacement by the *l*-stereoisomer.

DISCUSSION

It has recently been demonstrated (3) that β-receptor-activated adenylate cyclase in a number of cell types exhibits strict stereospecificity for *l*-catecholamines. It was found that *d*-catecholamines are unable to activate adenylate cyclase and also do not inhibit enzyme activation by the *l*-isomer. It has also been shown that only *l*-propranolol is capable of binding the β-receptor. *d*-Propranolol does not bind and does not interfere with the β-blocking activity of *l*-propranolol.

In this study, we have measured the potency of *d*- and *l*-catecholamines in displacing [^{3}H]propranolol specifically bound to the β-adrenergic receptor of turkey erythrocyte ghosts. We have used [^{3}H]propranolol because it was found to measure the β-receptor quantitatively and exclusively (1).

Using this technique, we have demonstrated the absolute stereospecificity for the *l*-isomers of catecholamines and propranolol. Furthermore, we have found that the *d*-isomers neither bind nor interfere with the binding of the *l*-isomers.

The dissociation constant measured for *l*-isoproterenol by the displacement assay is identical to the *apparent* dissociation constant measured for that ligand, using its activation of adenylate cyclase (Table 1). Similarly, the dissociation constants for propranolol measured by inhibition of adenylate cyclase and by direct binding measurements were also found to be identical (Table 1). Surprisingly, the binding occurs with a greater affinity than would be indicated by the activation of adenylate cyclase in the presence of these ligands. This discrepancy, although small, suggests that ATP, which is present in the activation experiments but is not present in the binding experiments, may modulate the affinity.

TABLE 1. *The dissociation constants of catecholamines*

Ligand	K_{Diss}, M	
	Kinetics of activation	binding
dl-Propranolol	2.5×10^{-9}	2.5×10^{-9}[a]
l-Propranolol	—	3.0×10^{-9}
d-Propranolol	No inhibition	No binding
l-Isoproterenol	5.0×10^{-7}	1.2×10^{-7}
l-Norepinephrine	3.0×10^{-6}	0.9×10^{-6}
l-Epinephrine	6.0×10^{-6}	2.0×10^{-6}
l-Epinephrine[b]	—	1.1×10^{-6}
d-Epinephrine	No activation	No binding

[a] From direct binding measurements (1).

[b] *l*-Epinephrine and *d*-epinephrine were mixed such that the concentration of *d*-epinephrine was three times the concentration of *l*-epinephrine at each point.

—, not done.

One may now quantitate and characterize the β-adrenergic receptor by this direct binding technique. Furthermore, one may now examine the effects of various adenylate cyclase effectors, such as nucleotides and cations, on the binding properties of the β-receptor. We find for example, that the presence of the allosteric inhibitor of adenylate cyclase Ca^{2+} (5) and the allosteric activator Gpp(NH)P (6) which potentiate *l*-catecholamine-stimulated adenylate cyclase, do not alter the affinity of the receptor towards *l*-propranolol (Levitzki *et al.*, unpublished).

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