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A Comparison of the *Beta*-Adrenergic Receptor of the Turkey Erythrocyte with Mammalian $Beta_1$ and $Beta_2$ Receptors

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SUMMARY

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The kinetic and pharmacological properties of β-adrenergic receptors in turkey erythrocyte membranes have been characterized by measuring adenylate cyclase activity and specific binding of ^{125}I-iodohydroxybenzylpindolol (IHYP). Receptor properties have been compared to those of β_1 and β_2 receptors in a number of mammalian tissues. The affinity (K_D) of IHYP for the turkey erythrocyte β-adrenergic receptor (42 pM) was similar to the K_D for IHYP binding to either β_1 or β_2 receptors. However, the rates of both association (k_1) and dissociation (k_{-1}) of IHYP were 6-10 times faster when measured with β-adrenergic receptors on turkey erythrocytes than were observed for either β_1 or β_2 receptors in various mammalian tissues. Thermodynamic analysis of the k_1 for IHYP binding in the turkey erythrocyte and rat heart showed similar enthalpies of activation ($\Delta H‡$), suggesting that the different k_1 values arise mainly from different entropies of activation ($\Delta S‡$) in the two tissues. The order of potency of drugs for activation or inhibition of adenylate cyclase activity correlated well with that for inhibition of IHYP binding in the turkey erythrocyte. However, both the K_i and K_{act} values for adenylate cyclase were generally two to three times higher than the corresponding K_D value determined from studies of the inhibition of IHYP binding. The pharmacological effects of a variety of drugs with similar or different affinities for β_1- and β_2-adrenergic receptors were determined on membranes prepared from turkey erythrocytes. The K_D values for nonselective drugs in the turkey erythrocyte were identical to their K_D values for β_1 and β_2 receptors, suggesting that this receptor should be classified as a β-adrenergic receptor. However, the K_D values in the turkey erythrocyte for selective drugs did not correlate with the K_D values for these drugs at either β_1 or β_2 receptors. Furthermore, the efficacies of partial agonists at turkey erythrocyte β-adrenergic receptors did not correlate with their efficacies for either β_1 or β_2 receptors. The results demonstrate that the β-adrenergic receptor in the turkey erythrocyte has kinetic and pharmacological properties distinct from either mammalian β_1 or β_2 receptors.

INTRODUCTION

Lands *et al.* (1) proposed that β-adrenergic receptors be divided into two subtypes based on differences in the pharmacological specificity of receptor mediated responses. β_1-Adrenergic receptors have similar affinities for epinephrine and norepinephrine and are found mainly in cardiac and adipose tissue. β_2-Adrenergic receptors have a much lower affinity for norepinephrine than for epinephrine and are found mainly in smooth muscle and in skeletal muscle and liver. Although there is some controversy concerning the general nature of this subdivision (2, 3) several lines of evidence support its validity (4, 5). Studies of β-adrenergic receptor subtypes have been, for the most part, performed on mammalian tissues.

We have recently presented evidence based on *in vitro* radioligand binding experiments for the existence of only two subtypes of β-adrenergic receptor in a variety of mammalian tissues. These tissues include heart, lung, liver and various brain regions in the rat; right atrium, left ventricle, and soleus muscle in the cat; and guinea pig heart (6-8). Taking advantage of the *in vitro* selectivity of certain drugs, a computer-based graphic method for quantitating the relative concentrations of β_1- and β_2-adrenergic receptors in tissues containing both recep-

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tor subtypes has been developed (7, 9). Using this method, we have shown that the pharmacological specificities of β_1- and β_2-adrenergic receptors are virtually identical for 17 drugs in 13 different mammalian tissues (8).[2] We have also shown that there is no change in the pharmacological specificity of these receptor subtypes in rat cerebral cortex and cerebellum during ontogeny.[3]

The β-adrenergic receptor of the turkey erythrocyte has frequently been used as a model system for studying β-adrenergic receptors and their coupling to adenylate cyclase (10–12). Based on the observation that epinephrine and norepinephrine have approximately equal affinities for this receptor (10, 11), the turkey erythrocyte β-adrenergic receptor has been classified as a β_1 receptor. Examination of the literature, however, reveals certain discrepancies between the properties of the turkey erythrocyte β-adrenergic receptor and those expected of a β_1 receptor. For example, the K_D value for the β_1 selective antagonist practolol has been reported to be 10 μM in the turkey erythrocyte (11), which is an order of magnitude higher than has been observed for mammalian β_1-adrenergic receptors (8). We have consequently carried out a comprehensive analysis of the pharmacological specificity of the turkey erythrocyte β-adrenergic receptor. Detailed evidence is presented that this receptor cannot be classified as belonging to either subtype.

METHODS

Preparation of turkey erythrocyte membranes. Turkey erythrocyte membranes were prepared as described by Øye and Sutherland (13). Blood was collected in heparinized plastic bottles and mixed with 0.25 vol of 75 mM glucose, 70 mM citric acid (pH 5). An equal volume of buffered saline (0.154 M NaCl containing 10 mM Tris–Cl (pH 7.4) and 1 mM EDTA) was added and the mixture was centrifuged at 800*g* for 10 min. The plasma and buffy coat layers were removed and the cells were washed twice in buffered saline. The cells were then hemolyzed by addition of 4 vol of distilled water and centrifuged at 1000*g* for 15 min. The hemolysis procedure was repeated four times and the membranes, resuspended in a minimal volume of buffered saline, were stored frozen at −70°.

Heart membranes were prepared as previously described (6).

Adenylate cyclase assay. Adenylate cyclase activity was determined by measuring the conversion of α^{32}P-ATP to ^{32}P-cyclic AMP and isolating the product by a modification of the method of Salomon *et al.* (14) as previously described (6). The reaction was carried out in a final volume of 0.2 ml including turkey erythrocyte membranes (1–3 mg of protein), 10 mM Tris–maleate (pH 7.5), 5 mM cyclic AMP, 0.75 mM isobutylmethylxanthine, 5 mM $MgCl_2$, 0.5 mM EGTA, 0.25 mM ATP, 0.1 mg/ml creatine kinase, 10 mM creatine-PO_4, and 30 μM GTP. Reactions were carried out for 10 min at 37° and were stopped by addition of 0.8 ml of water containing 1 mM ATP and by boiling for 1.5 min. The assay was linear with tissue up to 4 mg protein.

β-Adrenergic receptor binding assay. Hydroxybenzylpindolol (15) was iodinated and IHYP[4] was purified to theoretical specific activity (2.2 Ci/μmol) as previously described (16, 17). An aliquot (0.15 ml) of turkey erythrocyte membranes (8–10 μg of protein) was incubated with IHYP (60,000 cpm), 0.09 M NaCl and 12 mM Tris-HCl, pH 7.5 in a total volume of 0.25 ml (6).

Samples were incubated for 15 min at 37°. Reactions were stopped by addition of 10 ml of 0.154 M NaCl in 10 mM Tris buffer (0°, pH 7.5) to each assay tube, and the samples were rapidly filtered through Schleicher and Schuell glass fiber filters. Each filter was washed with an additional 10 ml of buffer at 0° and radioactivity was determined in a gamma counter.

Specific binding of IHYP was defined as the amount of IHYP bound in the absence of competing ligand minus the amount bound in the presence of 30 μM *l*-isoproterenol. This concentration of 1-isoproterenol is one hundred times its K_D value and with observed Hill coefficients of 1.0 corresponds to occupancy of 99% of the receptors. Specific binding routinely represented 75% of total IHYP binding in membranes from heart and 95% of total binding in turkey erythrocyte membranes. Scatchard analysis of specific IHYP binding to turkey erythrocyte membranes yielded a maximal number of IHYP binding sites (325 ± 9 fmol/mg protein) and a dissociation constant (K_D) of 4×10^{-11} M.

Determination of IC_{50} values. To determine the potency of drugs in inhibiting IHYP binding, samples were incubated with various concentrations of each drug in buffered saline as described above. Since the concentration of IHYP (50 pM) was close to the K_D of IHYP (40 pM), the concentration of drug that inhibited 50% of the specific binding of IHYP was approximately twice the K_D value of the drug (18). K_D values were calculated as described below. IC_{50} values were calculated from Hofstee plots for the inhibition of specific IHYP binding, or the inhibition of isoproterenol stimulated adenylate cyclase activity. In all cases the Hofstee plots were linear with Hill coefficients between 0.95 and 1.05.

Determination of K_D, K_i and K_{act} values. K_D values for the inhibition of IHYP binding by the various drugs were calculated by the method of Cheng and Prusoff (18) using the equation

$$K_D = IC_{50}/(1 + S/K_m),$$

where IC_{50} = concentration of drug inhibiting IHYP binding by 50%; S = concentration of IHYP; and K_m = K_D value for IHYP determined by Scatchard analysis (19).

K_i values for the inhibition of isoproterenol-stimulated adenylate cyclase activity by various drugs were determined from the same equation where IC_{50} = concentration of drug necessary to inhibit isoproterenol stimulation by 50%; S = concentration of isoproterenol; and K_m = K_{act} value for the stimulation of adenylate cyclase by isoproterenol. K_{act} values were determined as the concentration necessary for half maximal activation of adenylate cyclase.

[2] Hedberg, A., K. P. Minneman, and P. B. Molinoff, *J. Pharmacol. Exp. Therap.*, in press.

[3] Pittman, R. N., K. P. Minneman and P. B. Molinoff, *Bn. Res.*, in press.

[4] The abbreviation used is: IHYP, ^{125}I-iodohydroxybenzylpindolol.

Calculation of rate constants. The rate constant of association, k_1, was determined from the slope of the first order approximation of the integrated reversible second order equation (20):

$$\ln(RL_e/(RL_e - RL)) = k_1 t(L_T(R_T/RL_e) - RL_e),$$

where RL = concentration of ligand–receptor complex at time t, RL_e = concentration at equilibrium, L_T = total concentration of ligand (bound plus free), and R_T = total concentration of ligand binding sites determined by Scatchard analysis. The rate constant of dissociation, k_{-1}, was calculated from the slope of the first order plot: $-h_{-1}k_{-1}t = \ln(RL/RL_e)$ where RL is the concentration of complex remaining at time t after the addition of 0.6 μM *d,l*-propranolol and RL_e the concentration of complex immediately prior to the addition of propranolol.

Calculation of thermodynamic parameters. Thermodynamic parameters were calculated from transition state theory (21). Energy of activation (E_a) was calculated from the slope of the Arrhenius plot ($\ln k_1$ vs $1/T$) where the slope equals $-E_a/R$. R is the gas constant (1.99 cal/mol-°K), T is the temperature (in °K), and k_1 is the rate of the reaction. Enthalpy of activation ($\Delta H‡$) was calculated from the equation $\Delta H‡ = E_a - RT$. Free energy of activation ($\Delta G‡$) was calculated from the equation $\Delta G‡ = -RT \ln k_1 + RT \ln(kT/h)$ where k is Boltzmann's constant and h is Planck's constant. Entropy of activation ($\Delta S‡$) was calculated from the equation $\Delta G‡ = \Delta H‡ - T\Delta S‡$. All calculations were done for 37° (310°K). Thermodynamic parameters of equilibrium constants were calculated from the Gibbs–Helmholz equation. Standard enthalpy (ΔH^0) was calculated from the slope of the van't Hoff plot ($\ln K$ vs $1/T$) where K is the equilibrium association constant ($1/K_D$) and the slope equals $-\Delta H°/R$. Standard free energy (ΔG^0) was calculated from the equation $\Delta G° = -RT \ln K$. Standard entropy (ΔS^0) was calculated from the equation $\Delta G° = \Delta H° - T\Delta S°$.

Drugs. The following drugs were generously provided as gifts: metoprolol and terbutaline sulfate (CIBA GEIGY); soterenol, sotalol and zinterol (MJ-9184-1; N-(5(2-((1,1,dimethyl-2-phenyl-ethyl)amino)-1-hydroxy-ethyl)-2-hydroxypheny-1)-monohydrochloride) (Mead Johnson); salbutamol and salmefamol 1-(4-hydroxy-3-hydroxymethylphenyl)-2-(4-methoxy-α-methylphenethyl-amino)-ethanol (Allen and Hanbury's); fenoterol (3,5 dihydroxy-α-((p-hydroxy-α-methyl phenethyl)-amino)-methyl)benzyl alcohol and metaproterenol sulfate (Boehringer–Ingelheim, Ltd.); pindolol and hydroxybenzylpindolol (Sandoz Pharmaceuticals); H 35/25 ((1-(4-methylphenyl)-2-isopropyl aminopropranolol) (Hassel); atenolol (ICI Americas); MK-950 (timolol; Merck, Sharpe and Dohme); 1-propranolol (Ayerst); butoxamine was purchased from Burroughs–Wellcome; and practolol was kindly provided by Dr. N. Voelkel. Other drugs and reagents were commercially available.

RESULTS

Kinetic characteristics of IHYP binding to turkey erythrocytes and rat heart. The kinetics of association and dissociation of IHYP with β-adrenergic receptors on membranes prepared from turkey erythrocytes and rat heart are consistent with a second order reaction between ligand and receptor (Fig. 1). Equilibrium dissociation constants (K_D's) determined by Scatchard analysis agreed reasonably well with those calculated from the kinetic data, and were very similar in the two tissues studied. At 37° the K_D for rat heart was very similar to the K_D for IHYP binding to turkey erythrocytes membranes (Table 1). Despite the similarity in K_D values, the rate constants of IHYP binding were very different in the two tissues (Fig. 1, Table 1). IHYP associated and dissociated 6–10 times faster from its binding sites on turkey erythrocytes than from those in cardiac membranes (Table 1).

Thermodynamics of IHYP binding to turkey erythrocytes and rat heart. To more precisely examine the

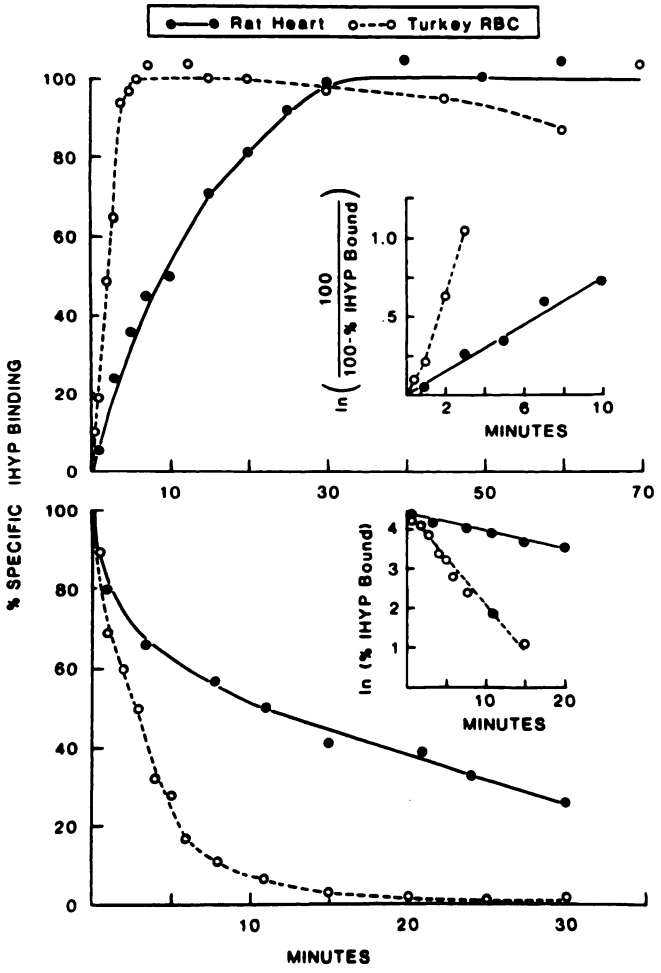

FIG. 1. *Kinetics of association and dissociation of IHYP binding to membranes from turkey erythrocytes and rat heart*

Upper: Association kinetics. Turkey erythrocyte membranes (≈11 pM in IHYP binding sites) or rat heart membranes (≈8 pM) were incubated with 20–26 pM IHYP as described in METHODS for the times indicated prior to filtration. Amount bound at equilibrium was 3.4 pM (erythrocytes) and 2.7 pM (heart). Inset: Pseudo-first order plot of association (see METHODS). Plots are linear up to or beyond 90% completion. Lower: Dissociation kinetics. Membranes (8–11 pM in IHYP binding sites) were equilibrated with 50 pM IHYP prior to the addition of 0.6 μM *d,l*-propranolol. Bound IHYP was determined at each time after propranolol addition as described in the text. Inset: First order plot of displacement.

TABLE 1

Rate and equilibrium constants for IHYP binding to turkey erythrocyte membranes and rat heart membranes[a]

Rate and equilibrium constants were determined as described in METHODS for IHYP binding to membranes prepared from turkey erythrocytes and rat heart. All measurements were done at 37°.

	k_1	k_{-1}	K_D	
			From Scatchard analysis	From kinetic constants
	(liters/mol-sec)	*(sec^{-1})*	(pM)	(pM)
Turkey erythrocyte	17.0×10^7	4.2×10^{-3}	43	24
Rat heart	1.6×10^7	0.7×10^{-3}	42	44

TABLE 2

Thermodynamic parameters of IHYP binding in turkey erythrocyte and rat heart

Thermodynamic parameters of rates (‡) and equilibrium (°) for IHYP binding to membranes from turkey erythrocytes and rat heart were determined as described in text.

	$\Delta G‡$	$\Delta H‡$	$\Delta S‡$
	(kcal/mol)	*(kcal/mol)*	*(entropy units)*
Turkey erythrocyte	+6.0	+17.7	+37.8
Rat heart	+7.5	+17.3	+31.2
	ΔG^0	ΔH^0	ΔS^0
	(kcal/mol)	*(kcal/mol)*	*(entropy units)*
Turkey erythrocyte	−14.8	−2.1	+40.9
Rat heart	−14.6	−2.7	+38.4

differences between the kinetics of IHYP binding in turkey erythrocytes and rat heart, a thermodynamic analysis was performed. Arrhenius plots for the association rate (k_1) in the two tissues were linear with almost identical slopes (Fig. 2), reflecting similar energies of activation (E_a). Calculation of the thermodynamic parameters of activation in the two tissues (Table 2) shows that the difference in the free energy of activation ($\Delta G‡$) reflected by the difference in rates is due almost exclusively to a difference in the entropy of activation ($\Delta S‡$) between the two tissues. Therefore, the faster rate of association of IHYP with turkey erythrocyte membranes is due to a larger positive entropy of activation. Calculation of the equilibrium thermodynamic parameters using a Van't Hoff plot revealed that these parameters are very similar in the two tissues (Table 2). The energy diagram using these thermodynamic parameters is shown in the inset to Fig. 2.

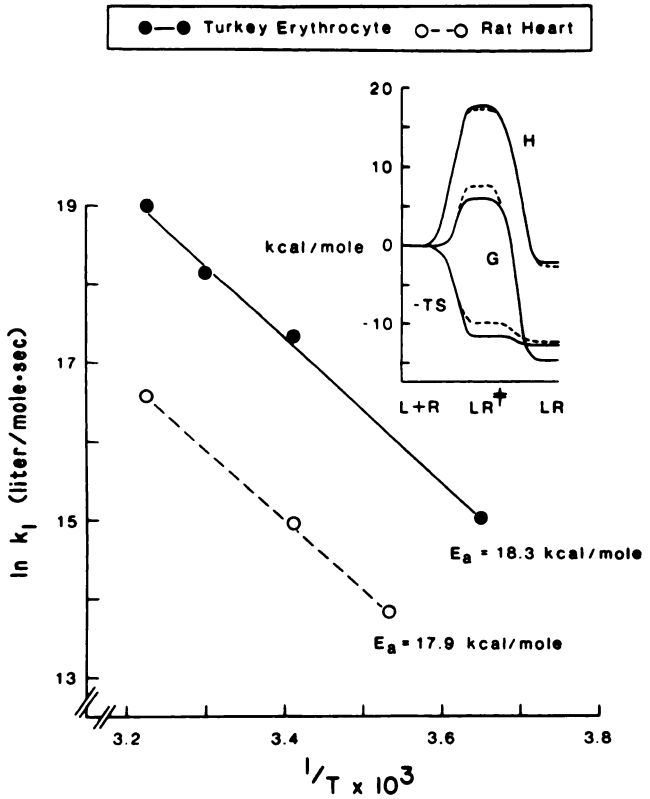

FIG. 2. *Arrhenius plot for association rate of IHYP binding to membranes from turkey erythrocyte and rat heart*

Rates of association (k_1) for IHYP binding were calculated as described in the text at three or four different temperatures. Rates were determined at two tissue concentrations or at two or three concentrations of IHYP. The standard error of the mean did not exceed 10%. Inset: Energy diagram for IHYP binding to turkey erythrocytes (——) and rat heart (- - -).

Pharmacologic comparison of β-adrenergic receptor-stimulated adenylate cyclase and inhibition of specific IHYP binding. To demonstrate that IHYP was binding to β-adrenergic receptors in turkey erythrocytes, the K_D values for displacing IHYP binding were compared to the K_i or K_{act} values for inhibiting or stimulating adenylate cyclase activity for a number of drugs (Fig. 3, Table 3). Although the K_D values were generally two to three times lower than the K_i values (Table 3), the pharmacological specificity of the two measurements were similar, and K_D values correlated very well with K_i or K_{act} values ($r^2 = 0.964$; Fig. 3).

Affinity of nonselective drugs for β-adrenergic receptors in turkey erythrocyte membranes. The K_D values in the turkey erythrocyte for a number of drugs that have been shown to have the same affinities for β_1- and β_2-adrenergic receptors (8) are listed in Table 4. Figure 4

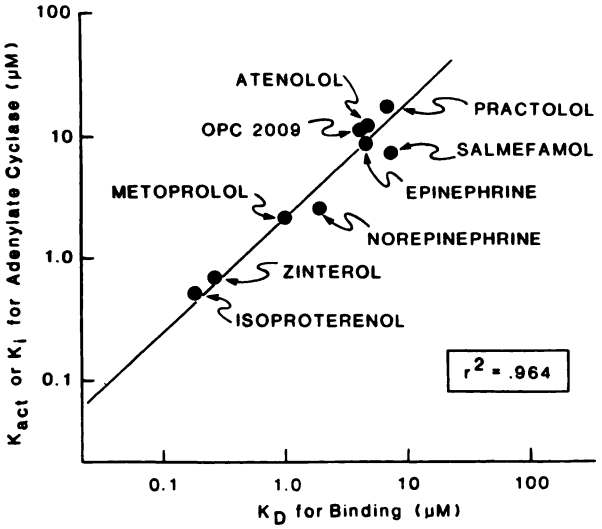

FIG. 3. *Correlation between K_D for IHYP binding and K_i or K_{act} for adenylate cyclase in turkey erythrocyte membranes*

Equilibrium constants were determined as described in text.

TABLE 3

Affinity of β_1 and β_2 selective drugs for β-adrenergic receptors in turkey erythrocyes

K_D and K_i values for IHYP binding and adenylate cyclase were determined as described in the text. Each value is the mean ± SEM of three experiments.

Drug	K_D	K_{act} or K_i
	(μM)	(μM)
β_1 Selective		
Practolol	6.9 ± 0.32	24.7 ± 1.37
Metoprolol	1.2 ± 0.17	3.1 ± 0.45
Atenolol	5.1 ± 0.09	11.0 ± 3.76
β_2 Selective		
Zinterol	0.36 ± 0.027	0.75 ± 0.038
Salmefamol	7.1 ± 0.43	8.7 ± 0.67
OPC 2009	4.6 ± 0.24	11.3 ± 1.13
IPS 339	0.0021 ± 0.00041	0.0096 ± 0.0032

TABLE 4

Affinities of nonselective drugs for β-adrenergic receptors in turkey erythrocytes

K_D values for IHYP binding were determined as described in the text. Each value is the mean ± SEM of three experiments.

Drug	K_D
	(μM)
Agonists	
l-Isoproterenol	0.26 ± 0.024
l-Epinephrine	5.0 ± 0.78
Soterenol	1.5 ± 0.07
Fenoterol	3.0 ± 0.23
Terbutaline	42 ± 6.9
Salbutamol	11 ± 0.8
Metaproterenol	16 ± 2.1
Antagonists	
l-Propranolol	0.0013 ± 0.00015
Sotalol	1.6 ± 0.08
Pindolol	0.0043 ± 0.00013
MK 950	0.0020 ± 0.00015
Butoxamine	3.7 ± 0.58
H 35/25	2.5 ± 0.43
H 133/22	0.41 ± 0.034

shows a comparison between the K_D values for these drugs for the turkey erythrocyte β-adrenergic receptor and the K_D values for β_1- and β_2-adrenergic receptors. The excellent correlation demonstrates that these drugs have the same affinity for the β-adrenergic receptor in the turkey erythrocyte as they do for β_1 and β_2 receptors in various mammalian tissues.

Affinities of β_1 and β_2 selective drugs for β-adrenergic receptors in turkey erythrocyte membranes. We have previously reported studies of a number of drugs having different affinities for β_1- and β_2-adrenergic receptors *in vitro* (8). Some of these drugs have a higher affinity for β_1 receptors (practolol, metoprolol, atenolol), while others have a higher affinity for β_2 receptors (zinterol, salmefamol, OPC 2009, IPS 339). The K_D values for these drugs in the turkey erythrocyte are listed in Table 3. All of the drugs tested resulted in linear Hofstee plots with Hill coefficients of around 1.0 for the inhibition of specific IHYP binding, suggesting that the β-adrenergic receptors in turkey erythrocyte membranes are of a homogeneous

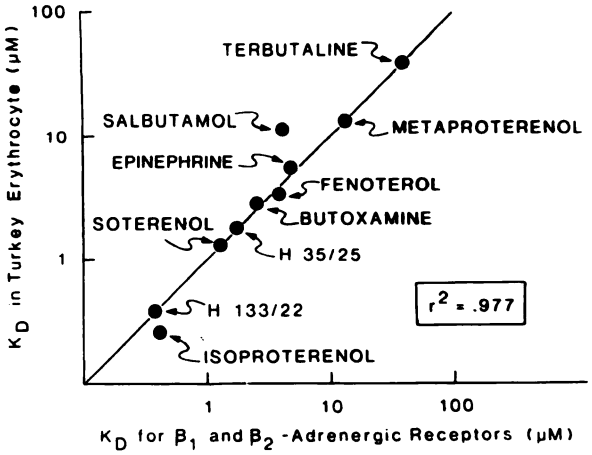

FIG. 4. *Correlation between the K_D values of nonselective drugs for IHYP binding sites in turkey erythrocytes and their K_D for β_1 and β_2 receptors*

K_D values for turkey erythrocyte membranes were determined as described in the text. K_D values for β_1 and β_2 receptors were taken from Ref. (8) and represent the mean value of K_D values for β_1 and β_2 receptors, which did not differ significantly in various rat, cat, and guinea pig tissues.

group. With the exception of salmefamol (which was a partial agonist), these drugs antagonized isoproterenol-stimulated adenylate cyclase activity in the turkey erythrocyte (Table 3).

The K_D values of these drugs for the turkey erythrocyte β-adrenergic receptor are compared with the K_D values which have been determined for β_1- and β_2-adrenergic receptors in a number of mammalian tissues in Fig. 5. It is clear that the K_D values for these drugs in the turkey erythrocyte do not agree with the K_D values for either β_1- or β_2-adrenergic receptors.

Efficacy of drugs in stimulating adenylate cyclase in turkey erythrocyte membranes. The effect of a variety of drugs that stimulate adenylate cyclase activity via mammalian β_2 receptors and are antagonists at β_1 receptors were examined in turkey erythrocytes. Salbutamol, salmefamol, soterenol, fenoterol, metaproterenol, terbutaline, and OPC 2009 stimulate adenylate cyclase activity in rat lung and liver and in guinea pig trachea, but have no effect on enzyme activity in heart or fat tissue (6, 22, 23). The efficacy of these drugs in stimulating adenylate cyclase activity in turkey erythrocytes are shown in Table 5. There was no correlation between the efficacies of these drugs in turkey erythrocytes and their efficacies at either β_1- or β_2-adrenergic receptors.

DISCUSSION

The β-adrenergic receptor of the turkey erythrocyte has been extensively used as a model system for studying the pharmacological properties of β-adrenergic receptors as well as for examining the coupling between receptors and adenylate cyclase (10–12). Since β-adrenergic receptors are not homogeneous (1, 8), it was important to define the similarities and differences between the receptor in turkey erythrocytes and the receptors in other tissues. Based on similar affinities of the endogenous catecholamines, epinephrine and norepinephrine, this re-

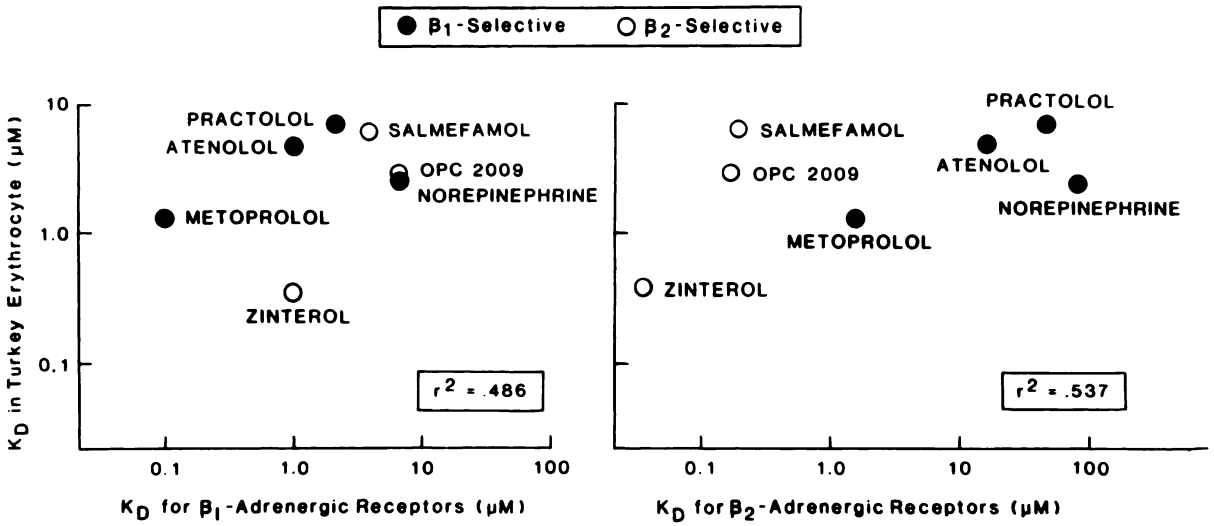

FIG. 5. *Correlation between K_D values of selective drugs for IHYP binding sites in turkey erythrocytes and their K_D for either β_1 or β_2 receptors*

Left: Correlation with β_1 receptors. Right: Correlation with β_2 receptors. K_D values for β_1 and β_2 receptors were taken from Ref. (8).

TABLE 5

Efficacy of agonists on β-adrenergic receptor stimulated adenylate cyclase in turkey erythrocytes

Drugs were tested for their efficacy in activating adenylate cyclase in the turkey erythrocyte. Isoproterenol, epinephrine and norepinephrine all activated adenylate cyclase to the same maximal extent. This activation was defined at 100%. The efficacy of these drugs on adenylate cyclase in rat heart and lung were taken from Ref. (6). GTP was present in all assays at a concentration of 30 μM.

Drug	Efficacy in turkey erythrocyte	Efficacy in rat heart	Efficacy in rat lung
	(%)	(%)	(%)
Salbutamol	0	0	58
OPC 2009	0	0	60
Terbutaline	15	0	27
Soterenol	35	0	70
Metaproterenol	43	0	60
Salmefamol	35	0	59
Fenoterol	53	45	100

ceptor has been considered to be an example of a β_1 receptor (10, 11). The evidence presented here shows that there are major kinetic and pharmacological differences between the β-adrenergic receptor in turkey erythrocytes and β_1 receptors in a variety of other tissues.

The association rate (k_1) for IHYP binding to β_1 and β_2 receptors in a number of tissues has been shown to be relatively constant. Thus, values of 0.67×10^7 liters/mol-sec for β_1 receptors in S49 lymphoma cells (24) and 1.1×10^7 liters/mol-sec for β_2 receptors in solubilized guinea pig lung (25) have been observed. We here report similar values for the k_1 value for IHYP binding to the receptors in rat heart, and we have observed that the rate of association of IHYP in rat cerebral cortex which has 81% β_1 receptors is very similar to that in rat heart (unpublished results). However, the k_1 for IHYP binding to turkey erythrocytes is much higher (17×10^7 liters/mol-sec), in agreement with results reported by Brown *et al.* (26). This difference is not due to the enrichment of receptor sites in the turkey erythrocytes, as the specific activity of IHYP binding sites was similar to that in S49 cells and solubilized guinea pig lung. It is clear, therefore, that the kinetics of IHYP binding to β-adrenergic receptors in turkey erythrocytes are very different from either β_1 or β_2 receptors. Since the dissociation rate (k_{-1}) is also much faster in the turkey erythrocyte, the equilibrium constant for IHYP is similar to that observed in other tissues.

Thermodynamic analysis of the difference between IHYP association rates in turkey erythrocytes and rat heart shows parallel Arrhenius plots, resulting in very similar enthalpies of activation ($\Delta H‡$). The differences in rates are due therefore to differences in the entropy of activation ($\Delta S‡$). Although interpretation of thermodynamic parameters in such a complex system is necessarily speculative, these measurements can suggest some ideas as to the differences between these receptors. Since the turkey erythrocyte β-adrenergic receptor has the same affinity for IHYP and a number of other nonselective ligands, as do β_1 and β_2 receptors, it seems likely that the recognition sites of these receptors are very similar. Also, the similarities in the enthalpies of activation for the β-adrenergic receptors in different tissues suggests that changes in the energy of intermolecular attractions or repulsions are similar for the different receptors. The larger positive $\Delta S‡$ for IHYP binding to the turkey erythrocyte might reflect a reduction in the steric hindrance (possibly reflecting a greater accessibility) for association and dissociation of the molecule from the receptor.

The affinities of β_1 and β_2 selective drugs for the β-adrenergic receptor in the turkey erythrocyte did not correlate with the affinity of these drugs for β_1- or β_2-adrenergic receptors as determined in a variety of mammalian tissues. This suggests that there must be small differences in the recognition sites of these different receptors. On the other hand, most of the drugs examined have the same affinity for β_1 and β_2 receptors in various tissues from rat, cat, and guinea pig (8). These drugs had

the same affinity for the β-adrenergic receptor in the turkey erythrocyte. It is clear from these observations that the fundamental properties of the recognition site that confer the β-adrenergic character of the receptor are conserved in all three of these receptors.

Although the properties of the β-adrenergic receptor of the turkey erythrocyte are different from those of either β_1 or β_2 receptors, it is not yet clear that it should be classified as a third subtype of β-adrenergic receptor. The major physiological difference between β_1 and β_2 receptors is their differential sensitivity to the endogenous catecholamines, epinephrine and norepinephrine. Therefore, although there are clearly differences in the physical properties of the receptors, in this respect, the turkey erythrocyte β-adrenergic receptor resembles the mammalian β_1 receptor. It may be that the differences between these two receptors result from small phylogenetic differences in the receptor.

The kinetic and pharmacological uniqueness of the turkey erythrocyte β-adrenergic receptor as described in this manuscript adds to the list of relatively unusual properties of this receptor. These properties include the lack of effect of guanyl nucleotides on agonist binding (11, unpublished results) and the absence of agonist-induced desensitization (27). It seems clear therefore, that the use of the turkey erythrocyte as a model system for studying β-adrenergic receptors should be approached with caution.

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