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Thermodynamics of Agonist and Antagonist Interactions with Mammalian β-Adrenergic Receptors

GREGORY A. WEILAND, KENNETH P. MINNEMAN, AND PERRY B. MOLINOFF

Department of Pharmacology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, Colorado 80262

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SUMMARY

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The thermodynamic parameters associated with the interactions of agonists and antagonists with β-adrenergic receptors on membranes prepared from rat cerebral cortex, cerebellum, heart, and lung were determined. The binding of [^{125}I]iodohydroxybenzylpindolol (IHYP) and the inhibition of IHYP binding by agonists and antagonists were examined at temperatures between 50 and 25°C. The density of receptors was not affected by the temperature at which the incubation was performed but the affinity of the receptor for agonists and antagonists increased as the temperature of the assay was decreased. The change in the affinity of the receptor for agonists was greater in magnitude than was the change in the affinity of the receptor for antagonists. The binding of antagonists was characterized by small decreases in enthalpy and substantial increases in entropy. The thermodynamic changes associated with agonist interactions with β-adrenergic receptors were characterized by large decreases in enthalpy and thermodynamically unfavorable decreases in entropy. In heart, lung, and cerebellum the affinities of agonists were decreased when assays were carried out in the presence of GTP. This nucleotide had no effect on the affinity of the β-adrenergic receptor in the cerebral cortex for agonists or for antagonists in any of the tissues examined. In tissues where binding of agonists was affected by the presence of GTP, the changes in enthalpy and entropy were less negative in the presence of GTP than in its absence.

INTRODUCTION

The use of radiolabeled antagonists such as iodohydroxybenzylpindolol (IHYP)[1] and dihydroalprenolol (DHA) has made it possible to carry out direct *in vitro* studies of β-adrenergic receptors. Such studies have led to an increasingly detailed knowledge of the kinetic and pharmacological properties of the receptor, of the localization of the receptor in a variety of tissues, and of the existence and properties of subtypes of this receptor (1–4). In addition, *in vitro* binding assays have permitted initial attempts to solubilize and partially purify this membrane-bound receptor so as to determine its chemical properties. However, the use of radioligands has not led to an understanding of the functional differences between agonists and antagonists. Although it is likely that agonists and antagonists bind to the same part of the receptor molecule, only agonists activate the enzyme adenylate cyclase.

We have recently examined the thermodynamics of ligand binding to β-adrenergic receptors on turkey erythrocyte membranes (5). The results showed that different driving forces were responsible for the binding of agonists and antagonists to the turkey erythrocyte β-adrenergic receptor. The binding of antagonists was almost totally entropy driven ($\Delta S° > 0$), while the binding of agonists was enthalpy driven ($\Delta H° < 0$) and was associated with large thermodynamically unfavorable decreases in entropy. The large decreases in enthalpy and entropy associated with agonist binding may reflect an agonist-induced conformational change in the receptor. The excellent correlation observed between the efficacy of full and partial agonists and the magnitude of the change in entropy and enthalpy are consistent with this possibility (5).

Recent evidence suggests that the β-adrenergic receptor of the turkey erythrocyte has different pharmacological and kinetic properties from β_1- or β_2-adrenergic re-

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[1] Abbreviations used: IHYP, iodohydroxybenzylpindolol; DHA, dihydroalprenolol; GMPPNP, guanylyl imidodiphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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ceptors in mammalian tissues (6). Furthermore, several investigators have reported that guanine nucleotides do not cause a decrease in the affinity of turkey erythrocyte β-adrenergic receptors for agonists (7, 8) though a decrease in affinity is observed in many mammalian systems (9, 10). Guanine nucleotide effects on agonist binding have recently been observed in studies of turkey erythrocyte β receptors following preincubation with GMP and isoproterenol (11). To determine whether the difference in the thermodynamic parameters associated with agonist as compared to antagonist binding to β-adrenergic receptors on turkey erythrocytes was unique to this tissue, the thermodynamics of ligand binding to β-adrenergic receptors in mammalian tissues have been determined. Since two subtypes of β-adrenergic receptor exist in mammalian tissues, four mammalian tissues were examined. The tissues chosen included tissues containing mostly β_1 receptors, most β_2 receptors, and tissues in which effects of GTP on agonist affinity for the receptor are or are not observed.

MATERIALS

The following compounds were generously provided as gifts: soterenol and sotalol (Mead Johnson), *l*-propranolol (Ayerst), phentolamine mesylate (CIBA-GEIGY), hydroxybenzypindolol (Sandoz Pharmaceuticals). Butoxamine was purchased from Burroughs-Wellcome, *l*-epinephrine and *l*-isoproterenol from Sigma Chemical Company. Guanosine triphosphate (GTP) and guanylyl imidodiphosphate (GMPPNP) were obtained from Boehringer-Mannheim. All other reagents were of the highest purity commercially available. [^{125}I]iodohydroxybenzylpindolol (IHYP, 2.2 Ci/μmol) was prepared and purified as previously described (12, 13).

METHODS

Preparation of membranes. Male Sprague-Dawley rats were sacrificed by decapitation and the hearts and lungs were removed, rinsed in 0.154 M NaCl containing 20 mM Na-Hepes, pH 7.5, (isosaline) and placed on ice. Brains were removed from the skull, and the cerebellum and cerebral cortex, dissected free of white matter, were rinsed in isosaline and placed on ice. Tissues were homogenized with a Brinkmann Polytron (setting 5–7) for 10–15 s in 20 vol of cold isosaline. Homogenates were centrifuged at 20,000*g* for 10 min and the supernatants decanted and discarded. After two resuspensions and centrifugations the "washed" pellets were resuspended in 100 (cerebellum), 133 (heart and cerebral cortex), or 500 (lung) volumes of isosaline.

Binding assay for β-adrenergic receptors. An aliquot (0.1 ml, 10–100 μg protein) of washed membranes was incubated with IHYP in a total volume of 0.25 ml containing 0.09 M NaCl, 1.1 mM ascorbic acid, 0.1 mM phentolamine, and 12 mM Na-Hepes (pH 7.3 at 25°C). Phentolamine, which reduced nonspecific binding by approximately 50%, did not affect the specific binding of IHYP (14, 15). Binding assays were routinely carried out in new disposable polypropylene tubes (Sarstedt). Samples were incubated for 10 min at 50°C, 20 min at 44°C, 45 min at 37°C, 1.5 h at 30°C, and 3 h at 25°C. Under these conditions the binding of IHYP was shown to reach equilibrium even in the presence of a competing agent. Reactions were stopped by the addition of 10 ml of 0.154 M NaCl in 10 mM Tris-HCl (25°C, pH 7.5) to each assay tube, and the samples were rapidly filtered through glass fiber filters (Schleicher and Schuell). Each filter was washed with an additional 10 ml of buffer and radioactivity was determined. 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 50 μM *l*-isoproterenol. Specific binding was 60–80% of total IHYP binding. Appropriate assay conditions were established to insure that less than 10% of the IHYP was bound.

The density of binding sites (B_{max}) and the equilibrium dissociation constant (K_d) for IHYP were determined from Scatchard analysis (16) of the amount of specifically bound IHYP at 6 concentrations of IHYP (30–250 pM). In other experiments samples were incubated with IHYP (routinely 45–55 pM) plus 14 concentrations of a competing drug. K_d values were then calculated from the concentration dependence of the inhibition of specific IHYP binding by the method of Cheng and Prusoff (17) using the equation

$$K_d = IC_{50}/(1 + L/K_m),$$

where IC_{50} = concentration of drug inhibiting specific IHYP binding by 50%, L = concentration of IHYP, and $K_m = K_d$ for IHYP determined by Scatchard analysis (see above). IC_{50} values were determined from Hill plots of the binding data. Hill coefficients for antagonists, including IHYP, and for agonists in the presence of GTP were approximately equal to 1.0.

Determination of thermodynamic parameters. Equilibrium thermodynamic parameters of binding were determined utilizing classical thermodynamic equations (18). The standard Gibbs free energy change ($\Delta G°$) of association was calculated from the equation $\Delta G° = RT \ln K_a$, where R is the gas constant (1.99 cal/mol-deg), T is the temperature in degrees Kelvin, and K_a is the equilibrium association constant ($1/K_d$). The standard enthalpy change ($\Delta H°$) was calculated from van't Hoff plots of the dependence of K_a on temperature between 25 and 50°C (298–323°K). The slope of a van't Hoff plot ($\ln K_a$ vs $1/T$) equals $-\Delta H°/R$. The standard entropy change ($\Delta S°$) was calculated from the equation $\Delta G° = \Delta H° - T\Delta S°$ after first determining $\Delta G°$ and $\Delta H°$ as described above. *The values reported in the tables are means ± SEM for three to six independent calculations on the thermodynamic parameters using individual tissue preparations.* All parameters reported are for 37°C (310°K). Hill coefficients for isoproterenol and epinephrine inhibition of IHYP binding to cerebellum, heart, and lung membranes in the absence of GTP were typically 0.5–0.7 which may reflect heterogeneity of binding sites for agonists, agonist-induced negative cooperativity, or a multistep binding reaction involving components in addition to agonist and receptor (19, 20). At the present time it is not possible to distinguish between these interpretations and thus the thermodynamic parameters are the average free energy, enthalpy, and entropy changes of the binding reaction, reflecting the apparent value of K_a at the midpoint of a binding isotherm (21).

RESULTS

Dependence of IC_{50} value on IHYP concentration. It was necessary to validate the Cheng and Prusoff (17) correction for K_d values, particularly where Hill coefficients were substantially less than one. IC_{50} values for the antagonist, sotalol, and the agonist, *l*-isoproterenol, were therefore determined in experiments using various concentrations of IHYP. The experiments with isoproterenol were carried out in the absence and in the presence of GTP. As shown in Fig. 1 the K_d of sotalol, calculated using the Cheng and Prusoff equation, was independent of the concentration of IHYP and agreed with the IC_{50} value extrapolated to the *y* intercept where the concentration of IHYP approaches zero. The K_d values for *l*-isoproterenol inhibition of IHYP binding in the absence or presence of GTP were also independent of the concentration of IHYP (Fig. 1). Therefore, even when the Hill coefficient for inhibition of IHYP binding is significantly less than 1.0, the Cheng and Prusoff equation can be applied to calculate a K_d value which reflects the concentration of ligand which saturates half of the binding sites.

The effect of pH on specific IHYP binding and on isoproterenol inhibition of IHYP binding to β-adrenergic receptors on lung membranes was examined since the temperature dependence of binding could reflect changes in the pK_a's of ionizable groups on the ligands or within the binding site. Examination of effects of pH were also undertaken to control for possible effects due to changes in the properties of the Na-Hepes buffer used in these experiments. As previously observed in studies of β-adrenergic receptors in rat cerebral cortex (22) and heart (13) binding of IHYP to lung membranes displayed a broad pH maximum (6.7–8.2) with no significant variations in the B_{max} or K_d. The K_d of isoproterenol was unchanged between pH 6.7 and 7.8 (data not shown). Therefore the effects observed were not due to changes in pK_a's of ionizable groups or to changes in the pH of the buffer.

Thermodynamics of IHYP binding to mammalian β-adrenergic receptors. IHYP binds to a single class of high-affinity sites in rat cerebral cortex, cerebellum, heart, and lung membranes. The affinity of these receptors for IHYP was reversibly increased by from 2.6- to 3.5-fold with no significant changes in B_{max}, as the temperature of the incubation was decreased from 50 to 25°C (Fig. 2). Van't Hoff plots of the dependence of IHYP affinity on temperature were linear over the temperature range examined for binding to cerebellum (Fig. 2, inset) and the three other tissues studied (data not shown). In other words, the enthalpy changes were independent of temperature and there was no evidence for changes in heat capacity during the binding reaction. Calculation of the thermodynamic parameters associated with the binding of IHYP revealed significant decreases in enthalpy and marked increases in entropy in all four of the tissues studied (Table 1). GTP (300 μM) had no effect on the B_{max} or K_d value or on the thermodynamics of IHYP binding (data not shown).

Thermodynamics of agonist interactions with mammalian β-adrenergic receptors. As observed in studies of turkey erythrocyte β-adrenergic receptors (5), the affinities of isoproterenol and epinephrine increased when the incubation temperature was decreased (Fig. 3; Table 2). These results were obtained with all four of the tissues studied. The temperature-dependent change in the affinity of isoproterenol was completely reversible and prior incubation at 50 or 25°C had no effect on the affinity determined in a subsequent incubation at a different temperature (data not shown). As previously reported (10), 300 μM GTP decreased agonist affinity and increased

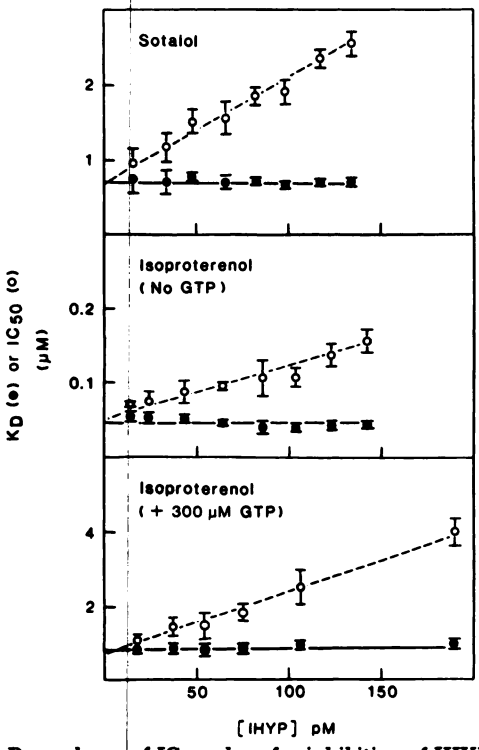

FIG. 1. *Dependence of IC_{50} values for inhibition of IHYP binding on the concentration of IHYP*

IC_{50} (○) and calculated K_d (●) values determined from inhibition of specific IHYP binding to membranes prepared from rat lung by sotalol (upper), isoproterenol in the absence of added GTP (middle), and isoproterenol in the presence of 300 μM GTP (bottom). K_d values were calculated as described under Methods assuming competitive interactions. *Values shown are means ± S.E.M. for three tissue preparations.*

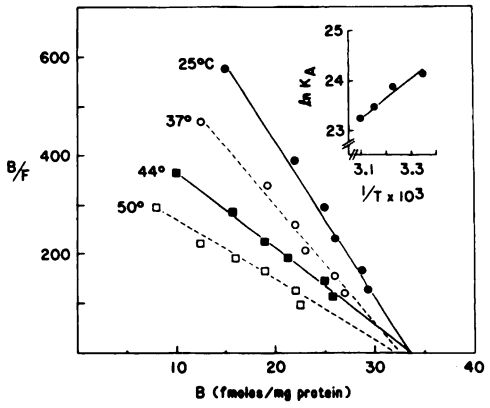

FIG. 2. *Scatchard analyses of the binding of IHYP to cerebellum membranes between 25 and 50°C*

Various concentrations of IHYP were incubated with membranes prepared from rat cerebellum at 50°C (□), 44°C (■), 37°C (○), and 25°C (●) as described under Methods. Inset: Van't Hoff plot of the dependence of K_a on temperature. The units of B/F are μl/mg.

TABLE 1

Equilibrium thermodynamic parameters of IHYP binding to β-adrenergic receptors

Values were determined as described under Methods and are means ± SEM *for three to six independent* determinations. The density of binding sites was 101 ± 1.3 fmol/mg protein for cerebral cortex, 33 ± 0.5 for cerebellum, 45 ± 1.5 for heart, and 263 ± 0.1 for lung membranes.

	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol-deg.)
Cerebral cortex	−14.4 ± 0.01	−9.5 ± 0.50	+15.7 ± 1.61
Cerebellum	−14.2 ± 0.03	−7.3 ± 1.31	+23.7 ± 4.86
Heart	−14.5 ± 0.09	−8.5 ± 0.65	+19.5 ± 2.34
Lung	−14.6 ± 0.08	−8.3 ± 0.96	+20.3 ± 2.09

the Hill coefficient for agonist inhibition of IHYP binding in rat heart, lung, and cerebellum, but not cerebral cortex. In studies carried out at 37°C with membranes prepared from rat heart and lung, 100 μM GMPPNP had the same effect on the affinity of isoproterenol as did 300 μM GTP (data not shown). In the presence of GTP, the affinities of the receptor for agonists were less sensitive to temperature in tissues (cerebellum, heart, and lung) where a significant effect of GTP on agonist binding was observed. In the cerebral cortex GTP had no effect on the affinities of the receptor for isoproterenol or epinephrine (10) (data not shown).

Van't Hoff plots for isoproterenol (Fig. 4) and epinephrine (data not shown) inhibition of IHYP binding were linear. The dependence of K_d on temperature was decreased in the presence of GTP. Thus the $\Delta H°$ was less negative in the presence of GTP than in its absence. The calculated thermodynamic parameters of isoproterenol and epinephrine interactions with mammalian β-adrenergic receptors are shown in Table 3. In all tissues, agonist binding was enthalpy driven and in most cases, the binding of agonists was associated with highly unfavorable decreases in entropy. In the presence of GTP, agonist binding was associated with less negative changes in both enthalpy and entropy (Table 3). The decrease in agonist affinity caused by GTP is thus primarily due to

TABLE 2

Equilibrium dissociation constants of agonist and antagonist binding to β-adrenergic receptors in rat lung

Dissociation constants were determined at 50 and 25°C. The results shown are means ± SEM *for three to six independent* determinations. In the absence of GTP, Hill coefficients for agonist binding were significantly less than 1.0; thus the values shown represent values of K_d at the midpoint of the binding isotherm.

	K_d (μM)		K_d (50°C)/ K_d (25°C)
	50°C	25°C	
Agonists			
l-Isoproterenol			
No GTP	0.217 ± 0.0290	0.0144 ± 0.0015*	15.1
+300 μM GTP	0.705 ± 0.0216	0.206 ± 0.0230*	3.42
l-Epinephrine			
No GTP	1.49 ± 0.238	0.277 ± 0.0306*	5.38
+300 μM GTP	7.72 ± 0.350	3.16 ± 0.316*	2.44
Antagonists			
l-Propranolol	0.0035 ± 0.000093	0.00214 ± 0.000154*	1.64
Sotalol	1.03 ± 0.035	0.854 ± 0.0544	1.21
Butoxamine	3.31 ± 0.211	2.82 ± 0.050	1.17

* $P < 0.05$.

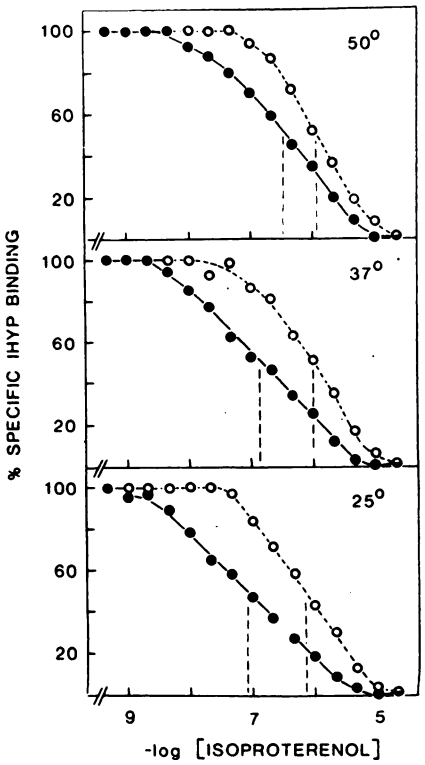

FIG. 3. *Concentration dependence of isoproterenol inhibition of IHYP binding to lung membranes in the presence and absence of GTP*

Membranes prepared from rat lungs were incubated with 50 pM IHYP and various concentrations of *l*-isoproterenol as described under Methods at 50°C (top), 37°C (middle), and 25°C (bottom) in the absence (●) or presence (○) of 300 μM GTP.

a less negative change in the enthalpy of binding despite the effect on the entropy change, which was in a direction that would contribute to an increase in the affinity of the receptor for agonists. GTP had no effect on the affinity of β-adrenergic receptors in the cerebral cortex for agonists (10). In this tissue the thermodynamic changes associated with the binding of agonists were unaffected by the presence or absence of GTP.

Thermodynamics of antagonist interactions with mammalian β-adrenergic receptors. The affinities of receptors in mammalian tissues for antagonists were relatively insensitive to changes in temperature. The K_d value of the high-affinity antagonist propranolol for mammalian β-adrenergic receptors changed a maximum of two fold between 50 and 25°C. The changes in affinity for the lower-affinity antagonists sotalol and butoxamine were even smaller. The enthalpy decreases associated with antagonist binding (−6.5 to −1.2 kcal/mol; Table 4) were small compared to those associated with the binding of agonists (−21.1 to −7.0 kcal/mol; Table 3). Antagonist binding was characterized by favorable entropy increases in marked contrast to the unfavorable entropy decreases associated with the binding of agonists. GTP had no effect on antagonist binding at any temperature examined (data not shown).

DISCUSSION

We have previously characterized the thermodynamic changes associated with the interactions of agonists and

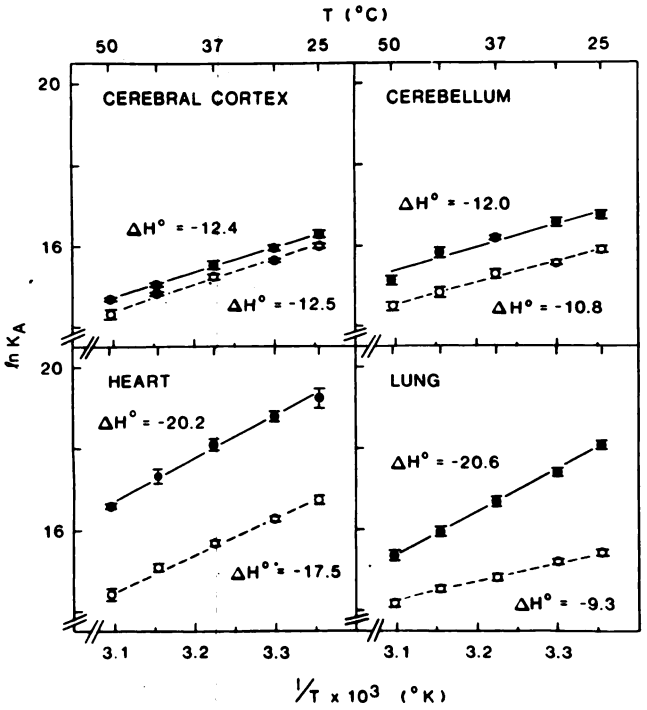

FIG. 4. *Van't Hoff plots for isoproterenol inhibition of IHYP binding*

Association constants for *l*-isoproterenol binding to membranes prepared from various rat tissues were determined between 25 and 50°C in the presence (○) and absence (●) of 300 μM GTP as described under Methods. The values shown are means ± SEM of ln K_a *for three to six determinations* at each temperature.

antagonists with turkey erythrocyte β-adrenergic receptors. Interpretation of data obtained with mammalian β-adrenergic receptors is complicated by the existence of two subtypes of β-adrenergic receptor and by the need to account for GTP-induced changes in the affinities of agonists. However, the results obtained in the present studies with mammalian β-adrenergic receptors are qualitatively similar to those we had previously obtained in studies of β receptors on turkey erythrocytes. Thus, antagonist binding to mammalian β-adrenergic receptors was associated with small decreases in enthalpy and substantial increases in entropy. The binding of antagonists has a large positive entropy component as has been observed for many other ligand–protein reactions where information transfer is not involved. Antigen–antibody reactions (23) and the binding of organic ions to bovine serum albumin (24) are examples of entropy-driven reactions that are associated with negligible contributions due to decreases in enthalpy. These reactions may be dependent on hydrophobic bonding, being driven by the increases in entropy which result when water molecules, ordered around hydrophobic components of the ligand and the binding site, are displaced (see (25)).

The thermodynamics of the binding of agonists to mammalian β-adrenergic receptors are significantly different from those of antagonists. Agonist binding is associated with substantial decreases in enthalpy which permit unfavorable decreases in entropy. The results presented here show that the thermodynamic parameters of agonist and antagonist binding are similar for tissues

TABLE 3

Equilibrium thermodynamic parameters of agonist interactions with rat β-adrenergic receptors

Values were determined as described under Methods and are means ± SEM *for three to six independent* determinations.

	ΔG° (kcal/mol)	ΔH° (kcal/mol)	ΔS° (cal/mol-deg)
Cerebral cortex			
l-Isoproterenol			
No GTP	−9.6 ± 0.08	−12.0 ± 0.50	−9.1 ± 1.98
+300 μM GTP	−9.4 ± 0.06	−12.5 ± 0.48	−9.7 ± 1.19
l-Epinephrine			
No GTP	—	—	—
+300 μM GTP	−7.8 ± 0.05	−11.8 ± 1.11	−12.9 ± 3.44
Cerebellum			
l-Isoproterenol			
No GTP	−10.0 ± 0.05	−12.2 ± 0.33	−7.4 ± 1.18
+300 μM GTP	−9.5 ± 0.05*	−11.3 ± 1.30	−5.8 ± 4.33
l-Epinephrine			
No GTP	−8.7 ± 0.05	−16.5 ± 0.83	−24.9 ± 2.56
+300 μM GTP	−8.2 ± 0.04*	−11.1 ± 0.83*	−9.4 ± 2.78*
Heart			
l-Isoproterenol			
No GTP	−11.2 ± 0.12	−21.1 ± 1.69	−32.0 ± 2.31
+300 μM GTP	−9.7 ± 0.03*	−17.2 ± 1.03*	−25.1 ± 3.28
l-Epinephrine			
No GTP	−8.9 ± 0.09	−14.6 ± 0.39	−18.5 ± 1.15
+300 μM GTP	−7.8 ± 0.05*	−10.9 ± 0.41*	−9.8 ± 1.40*
Lung			
l-Isoproterenol			
No GTP	−10.3 ± 0.07	−20.2 ± 1.09	−31.9 ± 3.71
+300 μM GTP	−9.1 ± 0.03*	−9.5 ± 0.55*	−1.0 ± 1.76*
l-Epinephrine			
No GTP	−8.8 ± 0.07	−12.6 ± 1.45	−12.1 ± 4.78
+300 μM GTP	−7.5 ± 0.3*	−7.0 ± 0.98*	+1.5 ± 3.09*

* $P < 0.05$, Student's *t* test.

containing primarily β_1-adrenergic receptors (cortex, heart) and tissues containing primarily β_2-adrenergic receptors (cerebellum, lung). In addition, these parameters are similar to those reported earlier for the turkey erythrocyte β-adrenergic receptors (5), which have kinetic and pharmacological properties different from those of mammalian β_1 or β_2 receptors (6). Thus the thermodynamic differences between agonist and antagonist binding may be a general characteristic of β-adrenergic receptors. It should be noted that the marked decrease in entropy observed in studies of agonist interactions with turkey erythrocyte β-adrenergic receptors is less pronounced in some mammalian tissues. Thus, only small changes in entropy were seen in the lung in the presence of GTP.

We have previously suggested that the substantial enthalpy decreases and unfavorable entropy decreases associated with agonist binding may reflect an initial binding reaction and a subsequent agonist-specific isomerization of the receptor to a conformation that can activate adenylate cyclase (5). Antagonists, which do not activate adenylate cyclase, would not induce a change in receptor conformation and thus would only participate in the initial binding reaction. The thermodynamics of antagonist binding reflect this initial binding reaction.

TABLE 4

Equilibrium thermodynamic parameters of antagonist interactions with rat β-adrenergic receptors

Values were determined as described under Methods and are means ± SEM *for three to six independent* determinations.

	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol-deg)
l-Propranolol			
Cerebral Cortex	−12.7 ± 0.003	−6.5 ± 0.21	+20.0 ± 0.68
Cerebellum	−12.8 ± 0.01	−5.7 ± 0.90	+22.4 ± 3.58
Heart	−13.2 ± 0.07	−6.2 ± 0.48	+22.7 ± 1.74
Lung	−12.3 ± 0.05	−4.2 ± 0.46	+26.0 ± 1.37
Sotalol			
Cerebral Cortex	−8.5 ± 0.03	−6.1 ± 0.30	+7.7 ± 0.81
Cerebellum	−8.5 ± 0.01	−2.7 ± 0.23	+18.8 ± 0.85
Heart	−8.3 ± 0.03	−5.9 ± 1.0	+7.8 ± 3.39
Lung	−8.6 ± 0.03	−1.3 ± 0.41	+23.7 ± 1.27
Butoxamine			
Cerebral Cortex	−7.8 ± 0.03	−1.3 ± 0.24	+20.0 ± 0.78
Cerebellum	−8.3 ± 0.08	−1.4 ± 0.35	+22.3 ± 0.89
Heart	−7.6 ± 0.01	−2.6 ± 1.04	+16.3 ± 3.32
Lung	−7.5 ± 0.06	−1.2 ± 0.09	+21.4 ± 1.20

Several recent findings are consistent with this hypothesis. The possibility that agonists cause changes in the conformation of the receptor is supported by reports of an agonist-induced increase in the apparent size of the β-adrenergic receptor (26, 27) and by the increased reactivity of the receptor toward *N*-ethylmaleimide following agonist exposure (28, 29). Direct evidence for a conformational change might be obtained from a kinetic analysis of agonist binding; however, studies with radiolabeled agonists have not been uniformly applicable. Kinetic analysis of antagonist binding ([^{125}I]IHYP and [^{3}H]DHA) is, in most cases, consistent with a simple second-order reaction (6, 12, 30). Gilman and co-workers (31) have, however, reported kinetic evidence for a two-step reaction of [^{125}I]IHYP with the β-adrenergic receptor of S49 lymphoma cell membranes.

Physicochemical interpretations of the entropy and enthalpy changes induced by agonists and the proposed conformational changes are necessarily speculative; however, a number of molecular events can be postulated which could account for the thermodynamic changes observed. Increased hydrogen or ionic bonding within the protein or between the protein and other components of the system (solvent water or the polar groups of lipids, for example) could lead to a decrease in enthalpy. The decreased entropy of the system may reflect a more restricted conformation of the protein or ordering of water around newly exposed regions of the protein. The decrease in entropy may even reflect receptor aggregation or interaction of the receptor with another membrane component such as the guanine nucleotide-binding protein (26, 27). An attractive possibility is that the decrease in entropy reflects an agonist-induced ordering of lipids surrounding the receptor. The effects of detergent solubilization on agonist affinity (32) and of the polyene antibiotics which are known to increase membrane fluidity on the coupling of β-adrenergic receptors with adenylate cyclase (33–35) make this a realistic possibility. The recent report on the thermodynamics of receptor-mediated activation of adenylate cyclase by prostaglandin E_1 and by increased acyl chain ordering (36) are also consistent with this speculation.

The most surprising result of these studies was the effect of GTP in tissues where agonist binding is affected by the presence of GTP. The decrease in agonist affinity caused by GTP could be due to a less negative $\Delta H°$ or to a more negative $\Delta S°$. Since GTP is required for the effective coupling of β-adrenergic receptors with adenylate cyclase (30) we had anticipated more negative entropy changes in the presence of GTP. However, the presence of GTP resulted in less negative changes in both enthalpy and entropy. It is likely that the decreases in enthalpy and entropy observed in the absence of GTP reflect a complex interaction between agonists and the receptor. Limbird and Lefkowitz (26, 27) have observed an agonist-induced increase in the Stokes radius of the receptor in the absence of guanine nucleotides. This increase appears to reflect interaction of the receptor with the guanine nucleotide-binding protein. The thermodynamic parameters of agonist binding in the absence of GTP may be a consequence of this structural change. Since adenylate cyclase activation by catecholamines appears to require GTP (31), these large enthalpy and unfavorable entropy decreases may reflect a conformational change in the receptor to a potentially active state which is not functionally expressed in the absence of GTP. Alternatively, the changes in entropy and enthalpy that occur in the absence of GTP may be associated with the process of desensitization. In any case, agonist binding is complicated in the absence of GTP by virtue of its being associated with low Hill coefficients.

In the cerebral cortex, GTP has no effect on the thermodynamics of isoproterenol binding and the values determined in this tissue for agonist binding in the absence of GTP are similar to those observed in GTP-sensitive tissues in the presence of GTP. Thus the lack of effects of GTP on isoproterenol binding in the cortex may be due to endogenous GTP bound to the regulatory site as has been shown recently for the turkey erythrocyte β receptor (11).

The fundamental differences between the thermodynamic changes associated with the binding of agonists as compared to antagonists may be a general property of adrenergic receptors. A temperature effect on the binding of agonists but not antagonists to α-adrenergic receptors of the calf brain has been reported (37); however, a thermodynamic analysis of binding was not described. A thermodynamic analysis has also been applied to some other receptor systems. Agonist and antagonist binding to the solubilized nicotinic receptor is characterized by surprisingly large increases in entropy and highly unfavorable increases in enthalpy (38). The physiological significance of these results, however, is unclear since in these studies the natural lipid environment of the receptor had been replaced by detergent. Recently Barlow and co-workers have thermodynamically characterized antagonist binding to muscarinic receptors and found the interactions to be entropy driven ($\Delta S° > 0$) with only slight and variable changes in enthalpy (39, 40). [1,2-$^{3}H_2$]Corticosterone binds to a receptor in rat thy-

mocytes with negative enthalpy and entropy components (41). The decrease in entropy presumably reflects changes in the conformation of the receptor molecule. Van't Hoff analysis of [^{3}H]flunitrazepam binding to benzodiazepine receptors exhibited two components. Below 16°C the binding was associated with a negative enthalpy change and a positive entropy change while above 16°C binding was totally enthalpy driven and was associated with slight decreases in entropy (42). Complex thermodynamics has also been reported for the interaction of insulin with its receptor (43). The enthalpy and entropy changes associated with the binding of insulin were very dependent on temperature; the enthalpy change was positive below 20°C and negative above 20°C. Entropy increased at all temperatures between 5 and 35°C reflecting an important hydrophobic component of the binding. Thus, as of the present time only a limited number of binding reactions have been examined thermodynamically. However, this approach should prove useful in characterizing drug–receptor interactions and increasing our understanding of receptor mechanisms.

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Send reprint requests to: Gregory A. Weiland, Ph.D., Department of Pharmacology, University of Colorado Health Sciences Center, 4200 E. Ninth Ave., Denver, Colo. 80262.