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COMMENTARY

Can Thermodynamic Measurements of Receptor Binding Yield Information on Drug Affinity and Efficacy?

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ABSTRACT. The present commentary surveys the methods for obtaining the thermodynamic parameters of the drug–receptor binding equilibrium, $\Delta G°$, $\Delta H°$, $\Delta S°$, and $\Delta C°_p$ (standard free energy, enthalpy, entropy, and heat capacity, respectively). Moreover, it reviews the available thermodynamic data for the binding of agonists and antagonists to several G-protein coupled receptors (GPCRs) and ligand-gated ion channel receptors (LGICRs). In particular, thermodynamic data for five GPCRs (β-adrenergic, adenosine A_1, adenosine A_{2A}, dopamine D_2, and 5-HT_{1A}) and four LGICRs (glycine, $GABA_A$, 5-HT_3, and nicotinic) have been collected and analyzed. Among these receptor systems, seven (three GPCRs and all LGICRs) show "thermodynamic agonist–antagonist discrimination": when the agonist binding to a given receptor is entropy-driven, the binding of its antagonist is enthalpy-driven, or vice versa. A scatter plot of all entropy versus enthalpy values of the database gives a regression line with the equation $T\Delta S°$ (kJ mol^{-1}; T = 298.15 K) = 40.3 (± 0.7) + 1.00 (±0.01) $\Delta H°$ (kJ mol^{-1}); N = 184; r = 0.981; $P < 0.0001$ – which is of the form $\Delta H° = \beta \cdot \Delta S°$, revealing the presence of the "enthalpy–entropy compensation" phenomenon. This means that any decrease of binding enthalpy is compensated for by a parallel decrease of binding entropy, and vice versa, in such a manner that affinity constant values (K_A) of drug–receptor equilibrium ($\Delta G° = -RT \ln K_A = \Delta H° - T\Delta S°$) cannot be greater than 10^{11} M^{-1}. According to the most recent hypotheses concerning drug–receptor interaction mechanisms, these thermodynamic phenomena appear to be a consequence of the rearrangement of solvent molecules that occurs during the binding. BIOCHEM PHARMACOL **60**;11:1549–1556, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. G-protein coupled receptors; ligand-gated ion channel receptors; binding thermodynamics; enthalpy–entropy compensation; agonist–antagonist discrimination; solvent reorganization

Only recently have the thermodynamic aspects of the equilibria of a great number of ligands to several GPCRs‖ and LGICRs been analyzed in sufficient detail. The significance of this approach can be appreciated by considering the more complete information on the drug–receptor interactions provided by a detailed thermodynamic analysis, with respect to the affinity constant measurements. In fact, the determination of drug–receptor binding constants (association, K_A, or dissociation, $K_D = 1/K_A$) by radiochemical specific binding assays makes it possible to calculate the standard free energy, $\Delta G° = RT \ln K_D$ (T = 298.15 K), of the binding equilibrium. This method is certainly an invaluable tool for the screening of potentially active drugs, the pharmacological characterization of receptor types and subtypes, and the identification of signal transduction pathways. On the other hand, the usual receptor binding assays provide little information on the molecular mechanisms underlying the interaction of a drug with a given receptor. In particular, if receptor binding experiments are performed at a single temperature, their results allow one to calculate the $\Delta G°$ of the equilibrium, but not its two components, as defined by the Gibbs equation $\Delta G° = \Delta H° - T\Delta S°$, where $\Delta H°$ and $\Delta S°$ are the equilibrium standard enthalpy and entropy, respectively. It can be assumed, in a simplified form, that these $\Delta H°$ and $\Delta S°$ terms represent the two classes of factors responsible for the drug–receptor recognition phenomenon [1]: non-bonded interactions such as hydrogen bonding and multipolar or dispersive interactions (which are related mainly to the enthalpic term), and solvent reorganisation (which can be associated with the entropic one). Moreover, it has been shown recently that the measurement of $\Delta H°$ and $\Delta S°$ values may be a simple *in vitro* method to discriminate drug effects on the signal

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‖ *Abbreviations:* GPCR, G-protein coupled receptor; LGICR, ligand-gated ion channel receptor; $\Delta C°_p$, standard heat capacity; $\Delta G°$, standard free energy; $\Delta H°$, standard enthalpy; $\Delta S°$, standard entropy; GABA, γ-aminobutyric acid; and EDF, equilibrium driving force.

transduction pathways. As a matter of fact, the agonist binding to a given receptor may be entropy-driven ($\Delta S° \gg 0$, $\Delta H° \geq 0$), while the antagonist binding is enthalpy-driven ($\Delta H° \ll 0$, $\Delta S° \leq 0$ or > 0), or vice versa. This phenomenon has been called "thermodynamic discrimination" [2].

For instance, thermodynamic measurements performed on the binding equilibrium of the adenosine A_1 receptor with a large number of agonists and antagonists have permitted researchers to establish that agonist binding is entropy-driven, whereas antagonist binding is essentially enthalpy-driven. A model of drug–adenosine A_1 receptor interaction has been proposed based on this behaviour, which accounts for the biological role of the moieties indispensable to confer agonist activity [2–4]. Moreover, recent research has demonstrated that the entropy-driven binding of agonists is greatly dependent on the presence of a Thr residue in the human adenosine A_1 receptor and that the enthalpy-driven binding of the antagonists does not show such a dependence [5].

Ten receptor systems have been studied so far from a thermodynamic point of view: five of these are GPCRs (adenosine A_1 [2–9], adenosine A_{2A} [10, 11], β-adrenergic [12, 13], dopamine D_2, [14–16], and 5-HT_{1A} [17, 18] receptors), four are LGICRs (glycine [19], $GABA_A$ [20, 21], 5-HT_3 [22, 23], and nicotinic [24–26] receptors), and one is a modulator (benzodiazepine receptor [27–29]).

The first case of thermodynamic discrimination was reported for the β-adrenergic receptor [12, 13], and only recently has it been confirmed for adenosine A_1 and A_{2A} receptors [2–5, 8, 9]. This phenomenon has not been observed for D_2 dopamine [14–16], 5-HT_{1A} [18], or benzodiazepine [27, 28] receptors. However, it has been proposed that thermodynamic analysis of benzodiazepine receptors and ligands, in the presence and in the absence of GABA, permits one to discriminate inverse agonists from antagonists and agonists [29]. The thermodynamic discrimination phenomenon also has been observed for glycine [19], $GABA_A$ [20], 5-HT_3 [22], and nicotinic [24–26] receptors.

These results indicate that not all the GPCRs studied discriminate their agonists and antagonists from a thermodynamic point of view and that this phenomenon appears to be typical for the four LGICRs examined.

This commentary reports the experimental methods needed for thermodynamic analysis of drug–receptor interactions. The results of this analysis applied to GPCRs and LGICRs are documented and discussed according to two important thermodynamic aspects: the agonist–antagonist discrimination and the enthalpy–entropy compensation phenomena.

METHODS OF THERMODYNAMIC MEASUREMENTS OF DRUG–RECEPTOR INTERACTIONS

GPCRs and LGICRs are membrane receptors, and, as a consequence, their concentrations are extremely low in biological tissues (typically 1–10 fmol/mg tissue) [30]. This situation has so far hampered any direct microcalorimetric determination of $\Delta H°$ for the drug–receptor equilibrium. Nevertheless, methods based on K_D measurements over a range of temperatures combined with van't Hoff analysis or other similar plots have been applied successfully to obtain the terms of the Gibbs equation. The general approaches used for affinity constant determination and for evaluation of thermodynamic parameters will be described.

Affinity Constant Determination

Binding assays are usually performed in the temperature range of 0–35°. Affinity constants are determined by means of two experimental procedures: saturation and inhibition experiments. The former are accomplished by incubating, at equilibrium, membranes of tissue or cell homogenates with increasing concentrations of radiolabelled ligand. For a generic binding equilibrium

$$L + R \rightleftarrows LR (L = \text{ligand}, \ = \text{receptor}),$$

affinity constants are calculated as $K_A = [LR]/([L][R]) = [LR]/[L_{max} - LR][B_{max} - LR] = 1/K_D$, where $[L_{max}]$ = total concentration of the ligand added, $[B_{max}]$ = total concentration of the binding sites, and K_D = dissociation constant. Since $[LR]/[L_{max} - LR] = [\text{Bound/Free}] = [B_{max}]K_A - K_A[\text{Bound}]$, the K_A and B_{max} values can be obtained from the slope and the intercept of the plot of [Bound/Free] versus [Bound] (Scatchard plot).

Inhibition experiments are performed by displacing a fixed concentration of radiolabelled ligand, [C*], from the receptor preparation with increasing concentrations of the unlabelled ligand under investigation, with the aim of determining its IC_{50}, that is, the inhibitor concentration displacing 50% of the labelled ligand. The affinity constant of the unlabelled drug, K_i, is subsequently calculated from the Cheng and Prusoff equation, $K_i = IC_{50}/1 + [C*]/K_D*$, where K_D* is the radioligand dissociation constant [31]; under controlled conditions $K_i = K_D = 1/K_A$. Another treatment consists in reconstructing mathematically the saturation curve of the unlabelled ligand from its inhibition curve, followed by the usual Scatchard plot analysis [32, 33].

Thermodynamic Parameter Determination

Equilibrium thermodynamic parameters can be calculated by two different methods:

METHOD A. The observed ΔG values ($\Delta G = -RT \ln K_A$) can be fitted by a quadratic expression [34]:

$$\Delta G° = A + BT + CT^2$$

It can be demonstrated that

$$\Delta H = \frac{\delta\left(\frac{\Delta G}{T}\right)}{\delta\left(\frac{1}{T}\right)_p} = A - CT^2;$$

$$\Delta S = -\left(\frac{\delta \Delta G}{\delta T}\right)_p = -B - 2CT;$$

$$\Delta C_p = -\left(\frac{\delta \Delta H}{\delta T}\right)_p = -2CT$$

(ΔC_p = equilibrium heat capacity difference)

Because experimental measurements are performed in a narrow range around $T° = 298.15$ K, equilibrium standard parameters $\Delta G°$, $\Delta H°$, and $\Delta S°$ are better obtained by interpolation in this range [35], i.e.

$$\Delta G° = A' + B'(T - T°) + C(T - T°)^2$$

for which

$$\Delta G° = A'; \quad \Delta H° = A' - B'T°; \quad \Delta S° = -B';$$
$$\Delta C_p^0 = -2C'T°$$

The condition $C = C' = 0$ (i.e. $\Delta C_p^\circ = 0$) corresponds to the case of a linear van't Hoff plot for which $\Delta H = \Delta H°$ and $\Delta S = \Delta S°$ at all temperatures. This condition is considered to be verified whenever the second-order C' coefficient is statistically not significant.

METHOD B. ΔC_p° can be assumed *a priori* to be essentially zero. This situation is not common in reactions involving biomacromolecules in solution but has been recognised frequently for the binding of drugs to membrane receptors [36]. In this case the van't Hoff equation, $\ln K_A = -\Delta H°/RT + \Delta S°/R$, gives a linear plot of $\ln K_A$ versus $1/T$. The standard free energy can be calculated from the van't Hoff plot of $\ln K_A$ versus $1/T$ as $\Delta G° = -RT \ln K_A$ at 298.15 K; the standard enthalpy can be calculated from the slope, $-\Delta H°/R$, and the standard entropy from the intercept, $\Delta S°/R$, or as $(\Delta H° - \Delta G°)/T$, with $T = 298.15$ K and $R = 8.314$ J K^{-1} mol^{-1}.

HOW CAN ΔG°, ΔH°, AND ΔS° DATA BE REPRESENTED?

Since $\Delta G°$ is related linearly to $\Delta H°$ and $\Delta S°$ by the Gibbs equation, $\Delta G° = \Delta H° - T\Delta S°$, it is useful to represent the thermodynamic data of drug–receptor interaction in a $-T\Delta S°$ versus $\Delta H°$ plot, as shown in Figs. 1 and 2. Several advantages can be achieved by this type of representation:

1. The plot allows one to obtain further information on $\Delta G°$ and, as a consequence, on K_A ($\Delta G° = -RT \ln K_A$). In fact, the same values of $\Delta G°$ (and therefore of K_A) can be produced by all the linear combinations of different $\Delta H°$ and $-T\Delta S°$ pairs of values lying on a diagonal of the plot. For example, the two dashed lines of the plots reported in Figs. 1 and 2 indicate the loci of the points corresponding to two different values of K_A at 25°C ($K_A = 10^4$ and 10^{11} M^{-1}), which represent, in approximate terms, the minimum and maximum values of K_A for the data of GPCRs and LGICRs reported in the present paper. All K_A values lie on parallel diagonal lines included between these two extremes.
2. As reported in Figs. 1 and 2, the $-T\Delta S°$ versus $\Delta H°$ plots show immediately the presence or the absence of the thermodynamic discrimination phenomenon between agonists (closed circles) and antagonists (open circles) for any given receptor.
3. $\Delta H°$ and $\Delta S°$ values of drug–receptor binding appear to be correlated according to the extrathermodynamic equation of the form $\Delta H° = \beta \cdot \Delta S°$ (β = constant) [36]. This phenomenon, called "thermodynamic compensation" or "enthalpy–entropy compensation," can be evaluated by linear regression analysis of $-T\Delta S°$ versus $\Delta H°$ plots. It will be described and discussed further for the data on GPCRs and LGICRs reported in this paper.

GPCRs

Table 1 reports the thermodynamic data for the five GPCRs which have been studied so far at a reasonable level of accuracy from a thermodynamic point of view. The ranges of $\Delta G°$, $\Delta H°$, and $-T\Delta S°$ values of both agonists and antagonist binding are given together with a qualitative classification of the prevailing EDF.

Only three of the five GPCRs reported in Table 1 are actually discriminated. This is clearly shown in Fig. 1, which summarizes in the form of $-T\Delta S°$ versus $\Delta H°$ plots the results of the thermodynamic measurements performed for each individual receptor system (data reported in the plots have been obtained by the references indicated in the last column of the tables).

As for the β-adrenergic receptor, agonists cluster in the exothermic region ($-143 \le \Delta H° \le -17$ kJ mol^{-1}) with negative or small positive standard entropy values ($-8 \le -T\Delta S° \le +93$ kJ mol^{-1}). Therefore, agonist binding is classified as enthalpy-driven. Conversely, the antagonist binding is mostly or totally entropy-driven ($-21 \le \Delta H° \le +16$ kJ mol^{-1}; $-53 \le -T\Delta S° \le -16$ kJ mol^{-1}).

Agonist binding at the adenosine A_1 receptor can be classified as totally entropy-driven ($+9 \le \Delta H° \le +50$ kJ mol^{-1}; $-106 \le -T\Delta S° \le -61$ kJ mol^{-1}), whereas antagonist binding is mostly or totally enthalpy-driven ($-44 \le \Delta H° \le -12$ kJ mol^{-1}; $-18 \le -T\Delta S° \le 7$ kJ mol^{-1}). A similar result, from a qualitative point of view, also has been obtained for the adenosine A_{2A} receptor: agonist binding is totally entropy-driven ($7 \le \Delta H° \le 50$ kJ mol^{-1}; $-83 \le -T\Delta S° \le -53$ kJ mol^{-1}), whereas antag-

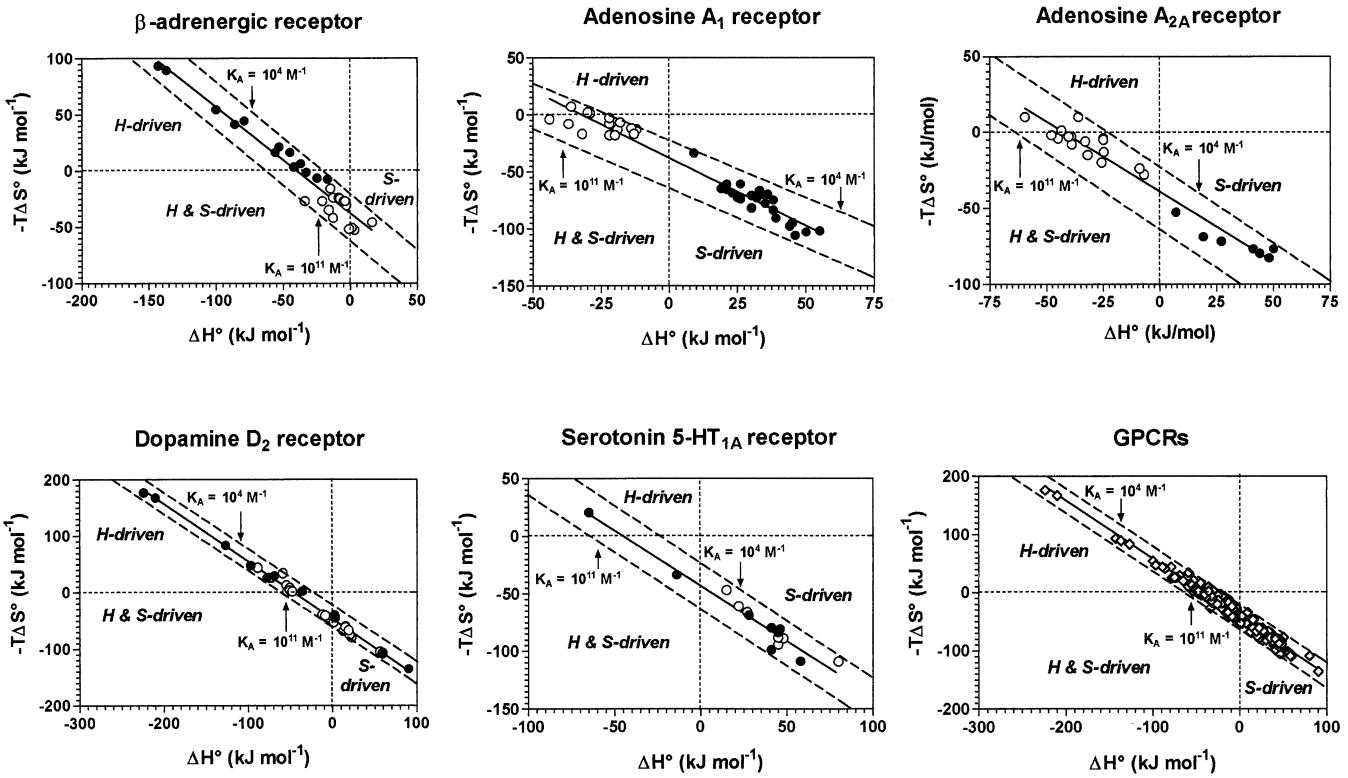

FIG. 1. $-T\Delta S°$ versus $\Delta H°$ scatter plots obtained from thermodynamic data of agonists (closed circles) and antagonists (open circles) binding to the GPCRs studied thus far. All the values reported on a common scatter plot (open rhombi) lie on the same regression line (continuous line) with the equation $T\Delta S°$ (kJ mol^{-1}) = 1.00 (± 0.01) $\Delta H°$ (kJ mol^{-1}) + 41.6 (± 0.8) ($N = 138$; $r = 0.985$; $P < 0.0001$). The means ± SD of slope and intercept values obtained for $-T\Delta S°$ versus $\Delta H°$ scatter plots of each individual receptor system are 1.01 ± 0.10 and 41 ± 4, respectively ($P < 0.0001$ and $0.972 \leq r \leq 0.996$). Diagonal dashed lines represent the loci of the points corresponding to two different values of K_A at 25° ($K_A = 10^4$ and 10^{11} M^{-1}).

onist binding is essentially enthalpy-driven ($-60 \leq \Delta H° \leq -7$ kJ mol^{-1}; $-28 \leq -T\Delta S° \leq +10$ kJ mol^{-1}).

Antagonist binding to the dopamine D_2 receptor can be enthalpy-driven, enthalpy- and entropy-driven, or entropy-driven ($-89 \leq \Delta H° \leq +59$ kJ mol^{-1}; $-105 \leq -T\Delta S° \leq +107$ kJ mol^{-1}), and similar behaviour is observed for the agonist binding ($-224 \leq \Delta H° \leq 90$ kJ mol^{-1}; $-136 \leq -T\Delta S° \leq +176$ kJ mol^{-1}). In this case, agonists and antagonists do not show the thermodynamic discrimination phenomenon.

Antagonist binding to the 5-HT_{1A} receptor is totally entropy-driven ($+15 \leq \Delta H° \leq +80$ kJ mol^{-1}; $-109 \leq -T\Delta S° \leq -47$ kJ mol^{-1}), but the agonist binding can be indifferently enthalpy-driven, enthalpy- and entropy-driven, or totally entropy-driven ($-65 \leq \Delta H° \leq +58$ kJ mol^{-1}; $-109 \leq -T\Delta S° \leq +20$ kJ mol^{-1}). Also in this case, therefore, agonists and antagonists cannot be discriminated thermodynamically.

A comprehensive analysis of all the thermodynamic data reported in Table 1 indicates that the total variation of $\Delta H°$ (-224 to $+90$ kJ mol^{-1}) and $-T\Delta S°$ values (-136 to $+176$ kJ mol^{-1}) is much greater than the variability of $\Delta G°$ values (-59.9 to -24.4 kJ mol^{-1}). This behaviour is at the root of the enthalpy–entropy compensation phenomenon [36]. In fact, any large decrease/increase of binding enthalpy is compensated by a parallel decrease/increase of binding entropy, in such a way that the standard free energy value ($\Delta G° = \Delta H° - T\Delta S°$) can remain confined within narrow limits. In particular, it is seen that the K_A values ($\Delta G° = -RT \ln K_A$) for the binding to GPCRs cannot exceed 10^{11} M^{-1}, the value represented in the plots by the lower dashed diagonal line.

Thermodynamic compensation is clearly shown by the lower right plot of Fig. 1, which shows the complete set of thermodynamic data for this family of receptors (GPCRs). The distance between the two dashed lines ($K_A = 10^4$ M^{-1} or $\Delta G° = -20$ kJ mol^{-1}; $K_A = 10^{11}$ M^{-1} or $\Delta G° = -60$ kJ mol^{-1}) can be considered a measure of total $\Delta G°$ variability in the case of GPCRs, and all data of the common $-T\Delta S°$ versus $\Delta H°$ scatter plot appear to be correlated according to the regression equation:

$$T\Delta S°\,(\text{kJ mol}^{-1}) = 41.6(\pm 0.8) + 1.00(\pm 0.01)\,\Delta H°\,(\text{kJ mol}^{-1})$$
$$(N = 138;\ r = 0.985;\ P < 0.0001) \qquad (1)$$

The data reported in the plots of any single GPCR appear similarly arranged along a diagonal line. In particular, the average ± SD of the slope and intercept values of regression lines are 1.01 ± 0.10 and 41 ± 4, respectively ($P < 0.0001$ and $0.972 \leq r \leq 0.996$). This result indicates that the $\Delta H°$

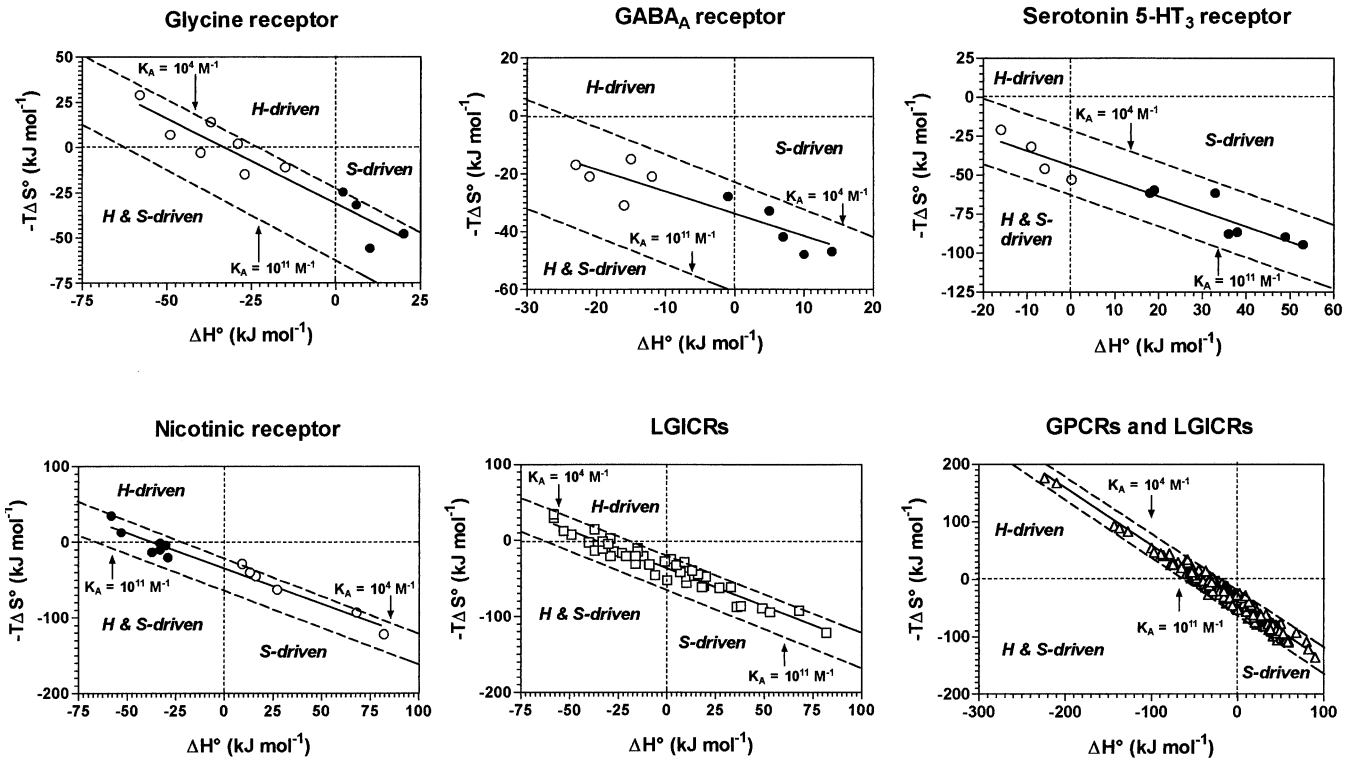

FIG. 2. $-T\Delta S°$ versus $\Delta H°$ scatter plots obtained from thermodynamic data of agonists (closed circles) and antagonists (open circles) binding to the LGICRs studied thus far. All the values reported on a common scatter plot (open squares) lie on the same regression line (continuous line) with the equation $T\Delta S°$ (kJ mol^{-1}) = 1.00 (± 0.04) $\Delta H°$ (kJ mol^{-1}) + 36.4 (± 1.4) (N = 46; r = 0.962, P < 0.0001). The mean ± SD of slope and intercept values, obtained from $-T\Delta S°$ versus $\Delta H°$ scatter plots of each individual receptor system, are 0.90 ± 0.09 and 36 ± 6, respectively (P < 0.001 and 0.886 ≤ r ≤ 0.975). The $-T\Delta S°$ versus $\Delta H°$ scatter plot of the overall data for GPCRs and LGICRs (open triangles) gives a regression line with the equation $T\Delta S°$ (kJ mol^{-1}) = 1.00 (± 0.01) $\Delta H°$ (kJ mol^{-1}) + 40.3 (± 0.7) (N = 184; r = 0.981, P < 0.0001). Diagonal dashed lines have the same meaning as described in the legend of Fig. 1.

and $-T\Delta S°$ data of the single GPCR systems are independently correlated on the same straight line.

LGICRs

Table 2 reports the thermodynamic data for the four LGICRs that have been studied so far at a reasonable level of accuracy from a thermodynamic point of view. Data are reported using the same system as in Table 1.

All the LGICRs discriminate the agonists from the antagonists, as shown in Fig. 2, which summarizes, in the form of $-T\Delta S°$ versus $\Delta H°$ plots, the results of the thermodynamic measurements performed in each individual system.

As for the glycine receptor, agonist binding is classifiable as entropy-driven ($2 \le \Delta H° \le +20$ kJ mol^{-1}; $-56 \le -T\Delta S° \le -25$ kJ mol^{-1}) and antagonist binding as mostly enthalpy-driven ($-58 \le \Delta H° \le -15$ kJ mol^{-1}; $-15 \le -T\Delta S° \le +29$ kJ mol^{-1}).

Agonist binding to the GABA$_A$ receptor is entropy-driven ($-1 \le \Delta H° \le +14$ kJ mol^{-1}; $-48 \le -T\Delta S° \le -28$ kJ mol^{-1}), whereas antagonist binding is both enthalpy- and entropy-driven ($-23 \le \Delta H° \le -12$ kJ mol^{-1}; $-31 \le -T\Delta S° \le -15$ kJ mol^{-1}). A similar result, from a qualitative point of view, has also been obtained for the 5-HT$_3$ receptor: the agonist binding is totally entropy-driven ($+18 \le \Delta H° \le +53$ kJ mol^{-1}; $-95 \le -T\Delta S° \le -60$ kJ mol^{-1}), whereas the antagonist binding is enabled by more favourable enthalpic contributions ($-16 \le \Delta H° \le 0$ kJ mol^{-1}; $-53 \le -T\Delta S° \le -21$ kJ mol^{-1}).

As far as the nicotinic receptor is concerned, agonist binding is essentially enthalpy-driven ($-58 \le \Delta H° \le -29$ kJ mol^{-1}; $-21 \le -T\Delta S° \le +34$ kJ mol^{-1}), whereas antagonist binding is totally entropy-driven ($9 \le \Delta H° \le 82$ kJ mol^{-1}; $-122 \le -T\Delta S° \le -29$ kJ mol^{-1}).

Also for LGICRs, an overall analysis of thermodynamic data indicates that the variability of $\Delta H°$ (−58 to +82 kJ mol^{-1}) and $-T\Delta S°$ (−122 to +34 kJ mol^{-1}) values is much greater than the variability of $\Delta G°$ values (−52.9 to −20.7 kJ mol^{-1}). The regression equation relating $\Delta H°$ and $-T\Delta S°$ values (see scatter plot in Fig. 2, LGICRs), is:

$$T\Delta S°(\text{kJ mol}^{-1}) = 36(\pm 1) + 1.00(\pm 0.04)\Delta H°(\text{kJ mol}^{-1})$$
$$(N = 46;\ r = 0.962;\ P < 0.0001) \qquad (2)$$

Data shown in the plots of any single LGICR appear similarly arranged along a diagonal line. In fact, the averages ± SD of slope and intercept values of regression

TABLE 1. Ranges of thermodynamic parameters, $\Delta G°$, $\Delta H°$, and $-T\Delta S°$, observed for the binding of agonists and antagonists to the five GPCRs so far studied

Receptor	N	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$-T\Delta S°$ (kJ mol^{-1})	EDF	References
β-Adrenoceptors						
Agonists	13	−50.6 to −25.9	−143 to −17	−8 to +93	*H*-driven	12, 13
Antagonists	15	−61.1 to −31.2	−21 to +16	−53 to −16	*S*- and *H*-driven	
Adenosine A_1						
Agonists	23	−59.9 to −24.7	+9 to +50	−106 to −61	*S*-driven	2, 3, 6, 7, 9
Antagonists	16	−49.2 to −24.4	−44 to −12	−18 to +7	*H*- and *S*-driven	
Adenosine A_{2a}						
Agonists	7	−50.2 to −27.2	+7 to +50	−83 to −53	*S*-driven	10, 11
Antagonists	16	−50.2 to −26.2	−60 to −7	−28 to +10	*H*- and *S*-driven	
Dopamine D_2						
Agonists	11	−53.2 to −33.9	−224 to +90	−136 to +176	none discriminated	14, 15, 16
Antagonists	22	−59.0 to −24.3	−89 to +59	−105 to +107	none discriminated	
5-HT_{1A}						
Agonists	8	−58.3 to −35.8	−65 to +58	−109 to +20	none discriminated	18
Antagonists	7	−49.0 to −28.8	+15 to +80	−109 to −47	none discriminated	

N = number of ligands; T = 298. 15 K; EDF = equilibrium driving force.

lines are 0.90 ± 0.09 and 36 ± 5, respectively ($P < 0.001$ and $0.886 \leq r \leq 0.975$).

These results suggest for LGICRs the presence of the same extrathermodynamic compensation mechanism observed for GPCRs. The overall $-T\Delta S°$ versus $\Delta H°$ scatter plot of the overall data for GPCRs and LGICRs (lower right plot of Fig. 2) gives a regression line with the equation

$$T\Delta S°(\text{kJ mol}^{-1}) = 40.3(\pm 0.7) + 1.00(\pm 0.01)\Delta H°(\text{kJ mol}^{-1})$$
$$(N = 184;\ r = 0.981;\ P < 0.0001) \qquad (3)$$

which is not significantly different from the regression lines reported in Figs. 1 and 2. In fact, the averages ± SD of their slopes and intercepts are 0.97 ± 0.10 and 39 ± 5, respectively.

DISCUSSION

The regression equation (3) has been obtained by plotting standard enthalpy and entropy data of 184 independent experiments performed on nine different membrane receptor systems, belonging to the GPCR and LGICR families. This equation is of the form $\Delta H° = \beta \cdot \Delta S°$, which is expected for a case of enthalpy–entropy compensation [35–39] with a compensation temperature β of 298 K. The correlation confines all affinity constant values in the region between the two diagonal dashed lines marked as $K_A = 10^4$ and $K_A = 10^{11}$ M^{-1} in all $-T\Delta S°$ versus $\Delta H°$ plots of Fig. 1 and 2. It must be stressed, however, that the meanings of the upper and lower limits are quite different. The upper one is due to the intentional exclusion of drugs with K_D values greater than 100 μM because they cannot be of practical use. Conversely, the lower diagonal line reflects a true physicochemical constraint to which membrane receptors and their ligands appear to conform in the binding process, that is, their K_D values never become smaller than approximately 10 pM. Extrathermodynamic enthalpy–entropy compensation properties, which seem to be common to most, if not all, biological macromolecular

TABLE 2. Ranges of thermodynamic parameters, $\Delta G°$, $\Delta H°$, and $-T\Delta S°$, observed for the binding of agonists and antagonists to the four LGICRs so far studied

Receptor	N	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$-T\Delta S°$ (kJ mol^{-1})	EDF	References
Glycine						
Agonists	4	−47.50 to −23.8	+2 to +20	−56 to −25	*S*-driven	19
Antagonists	7	−43.5 to −22.50	−58 to −15	−15 to +29	*H*- and *S*-driven	
$GABA_a$						
Agonists	6	−40.4 to 29.9	−1 to +14	−48 to −28	*S*-driven	20
Antagonists	5	−47.8 to 30.4	−23 to −12	−31 to −15	*H*- and *S*-driven	
5-HT_3						
Agonists	7	−52.3 to −28.4	+18 to +53	−95 to −60	*S*-driven	22
Antagonists	4	−52.9 to −37.2	−16 to 0	−53 to −21	*H*- and *S*-driven	
Nicotinic						
Agonists	7	−50.7 to −25.0	−58 to −29	−21 to +34	*H*- and *S*-driven	24, 25, 26
Antagonists	6	−36.8 to −20.73	+9 to +82	−122 to −29	*S*-driven	

N = number of ligands; T = 298.15 K; EDF = equilibrium driving force.

systems, have been discussed in some detail by different authors [36, 38–40] and are generally ascribed to solvent reorganisation that accompanies the receptor binding processs. Moreover, an elegant interpretation of the entropy-driven binding mechanisms has been proposed, evaluating the possible role of "entropic trapping" in receptor–ligand systems [41, 42].

Recently, a general model has been proposed for which every association process involving rearrangement of water molecules must be in thermodynamic compensation (if the reacting systems are very dilute, as is the case for drug–receptor interactions), with a compensation temperature not far from the experimental one [39]. According to this model, the solvent molecules do not affect the intrinsic affinity constant (K_A) of the drug–receptor interaction because the standard free energy for solvent reorganization, $\Delta G°_{SR}$, is normally zero. On the other hand, they can consistently affect, according to the molecular mechanism which permits the binding itself, the standard enthalpy and entropy ascribed to solvent reorganization, $\Delta H°_{SR}$ and $\Delta S°_{SR}$, which turn out to be related by the equation $\Delta H°_{SR} - T\Delta S°_{SR} = \Delta G°_{SR} = 0$. In other words, while $\Delta G°$ (or K_A) binding values most probably are determined by the specific features of the ligand and receptor undergoing the binding process, the interrelated $\Delta H°$ and $-T\Delta S°$ values are to be attributed mostly to the rearrangements occurring during the binding, in the solvent or, better, at both solvent–drug and solvent–receptor interfaces [39].

Previous considerations suggest that solvent effects might be responsible for the *in vitro* thermodynamic discrimination between agonists and antagonists observed for all LGICRs and some of the GPCRs studied thus far. However, it is not easy to understand why agonists and antagonists may be discriminated thermodynamically, i.e. located in two different regions of the enthalpy–entropy compensation band, when the compensation itself is to be ascribed to a simple rearrangement of water molecules bearing little relationship with either the binding affinity or the intrinsic activity. This problem has been debated already for both β-adrenergic [12] and adenosine A_1 receptors [2], both belonging to the class of GPCRs. In the case of the adenosine A_1 receptor, the entropy-driven binding of agonists has been interpreted by a model of drug–receptor interaction accounting for the biological role played by a ribose ring, the moiety indispensable to confer agonist activity on adenosine A_1 receptor ligands and absent in antagonists. It was suggested that the ribose ring can dock in a pocket of the binding site previously filled by a network of water molecules, thus releasing a large number of solvent molecules, which account for the observed entropy increase. At the same time, the insertion of the ribose moiety and the depletion of the water network induce conformational changes in the receptor, which are able to trigger the final agonist effect [2, 3]. Such a model implies that both affinity and efficacy of adenosine receptor ligands are related. This was confirmed by revealing a high correlation between intrinsic activity and values of $\Delta S°$ (which constitute the driving force of the agonist–receptor interactions) for a wide variety of adenosine A_1 receptor ligands acting as full or partial agonists and as antagonists. Standard entropy values therefore have been proposed as indicators of the pharmacological profile of A_1 ligands, in terms of their affinity and intrinsic activity [3, 4, 9].

A similar molecular mechanism has been proposed for LGICRs [26]. All known LGICRs are, in fact, thermodynamically discriminated (see Table 2 and Fig. 2), suggesting an interconnection between specific binding and abrupt variation of water-accessible receptor surfaces consequent to the setting up of the peculiar receptorial effect, that is, the channel opening.

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