ELSEVIER

Il Farmaco 53 (1998) 249–254

IL FARMACO

Receptor binding thermodynamics as a tool for linking drug efficacy and affinity [1]

Pier Andrea Borea [a,*], Katia Varani [a], Stefania Gessi [a], Paola Gilli [b], Alessandro Dalpiaz [c]

[a] *Dipartimento di Medicina Clinica e Sperimentale, Sezione di Farmacologia, Università di Ferrara, via Fossato di Mortara 19, 44100 Ferrara, Italy*

[b] *Dipartimento di Chimica, via Borsari 46, 44100 Ferrara, Italy*

[c] *Dipartimento di Scienze Farmaceutiche, via Fossato di Mortara 19, 44100 Ferrara, Italy*

Abstract

Determination of drug-receptor binding constants (association, K_A, or dissociation, $K_D = 1/K_A$) by radiochemical specific binding assays has proved to be an invaluable tool for screening of potential active drugs. Simple determination of K_A (or K_D) values makes it possible, however, to calculate the standard free energy $\Delta G^\circ = -RT \ln K_A = RT \ln K_D$ ($T = 298.15$ K) of the binding equilibrium but not that of its two components as defined by the Gibbs equation $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$, where ΔH° and ΔS° are the equilibrium standard enthalpy and entropy, respectively. This incomplete knowledge is highly inconvenient from a pure thermodynamic point of view as ΔH° and ΔS° carry much information on the details of the drug–receptor interaction and the interplay of both reaction partners with the solvent. In recent times it has been shown that the relative ΔH° and ΔS° magnitudes can often give a simple 'in vitro' way for discriminating 'the effect', that is the manner in which the drug interferes with the signal transduction pathways. This particular effect, called 'thermodynamic discrimination', results from the fact that binding of antagonists may be enthalpy-driven and that of agonists entropy-driven, or vice versa. The first case of thermodynamic discrimination was reported for the β-adrenergic G-protein coupled receptor (GPCR) and only recently has it been confirmed for adenosine A_1 and A_{2a} receptors. Only very recently has the binding thermodynamics of ligand-gated ion channel receptors (LGICR) been investigated and data for four receptors have been reported showing that all of them are thermodynamically discriminated. While it seems difficult at present to find a reasonable explanation for the thermodynamic discrimination phenomenon in GPCR, some hypotheses can be suggested for LGICR. Since global ΔH° and ΔS° values of the binding process are expected to be heavily affected by rearrangements occurring in the solvent, thermodynamic discrimination in LGICR is at least logically understandable admitting that the observed ΔH° (and then ΔS°) values are determined by both specific binding and abrupt variation of water-accessible receptor surfaces consequent to the setting up of the channel opening.

Keywords: Binding thermodynamics; G-protein coupled receptors; Ligand-gated ion channel receptors; Agonist–antagonist discrimination; Enthalpy–entropy compensation

1. Introduction

Determination of drug–receptor binding constants (association, K_A, or dissociation, $K_D = 1/K_A$) by radiochemical specific binding assays has proved to be an invaluable tool for screening of potential active drugs, pharmacological characterisation of receptor types and subtypes and identification of signal transduction pathways. However, the usual receptor binding assays performed at a single experimental temperature provide little information about the molecular mechanisms underlying the interaction of a drug with a given receptor. In fact, simple determination of K_A (or K_D) values makes it possible to calculate the standard free energy $\Delta G^\circ = -RT \ln K_A = RT \ln K_D$ ($T = 298.15$ K) of the binding equilibrium, but not its two components as defined by the Gibbs equation $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$. ΔH° and ΔS° are the equilibrum standard enthalpy and entropy, respectively. It can be assumed, in a very simplified form, that standard enthalpy is a quantitative indicator of the changes in intermolecular bond energies (hydrogen bonding and van der Waals interactions) occurring during the binding [1]. Standard entropy, on the other hand, can be considered an indicator of the rearrangements undergone by the solvent (water) molecules during the same process [1]. This lack of data is mostly due to the extremely low concentrations of receptors present in biological tissues (typically 1–100 fMol/mg of tissue for most neurotransmitter receptors [2]) which has so far made any

* Corresponding author. Tel.: +39-532-291-214; fax: +39-532-291-205; e-mail: bpa@dns.unife.it

[1] Presented at the First Italian–Swiss Meeting on Medicinal Chemistry, Turin, Italy, September 1997.

0014-827X/98/$19.00
PII S0014-827X(98)00017-2

microcalorimetric determination of ΔH° impossible. Nevertheless, a method based on K_D measurements over a range of temperatures combined with analysis of van't Hoff plots has been successfully applied to obtain the thermodynamic terms belonging to Gibbs equation. Critical appraisals of this method are available [3–6].

In recent times it has been moreover shown that the ΔH° and ΔS° values of drug interaction with a defined receptor can often give a simple 'in vitro' way to discriminate 'the effect', that is the manner in which the drug interferes with the signal transduction pathways. This particular effect has been called 'thermodynamic discrimination' [7] and results from the fact that binding of agonists may be entropy-driven and that of antagonists enthalpy-driven, or vice versa.

Ten receptor systems have so far been extensively studied from a thermodynamic point of view: among these, seven show the agonist–antagonist discrimination and three do not. The first case of thermodynamic discrimination was reported for the β-adrenergic G-protein coupled receptor (GPCR) [8,9] and only recently has it been confirmed for adenosine A_1 and A_{2A} receptors (GPCR) [7,10,11]. This phenomenon has not however been observed for two other GPCR (D_2-dopamine [12,13] and 5-HT_{1A} [14]) and a modulator one (benzodiazepines receptor [15,16]). All the ligand-gated channel receptors (LGICR) investigated from a thermodynamic point of view (glycine [17], $GABA_A$ [18], 5-HT_3 [19,20] and neuronal nicotinic [21]) have been reported to discriminate 'in vitro' the effect of their agonists and antagonists.

The results derived from the binding thermodynamic analysis of agonists and antagonists with GPCR and LGICR systems are briefly reviewed and discussed. The discussion is based on the capability of the receptor to show the thermodynamic discrimination phenomenon.

2. Methods and calculations

2.1. Affinity constant determination

Binding assays are usually performed in the temperature range 0–35°C. Affinity constants are determined by means of two experimental procedures: saturation and inhibition experiments. The former are accomplished by incubating at equilibrium fractions of tissue homogenates with increasing concentrations of radiolabelled ligand. For a generic binding equilibrium $L + R \rightleftarrows LR$ (L = ligand, R = 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 $[LR]/[L_{MAX} - LR] = [Bound/Free] = [B_{MAX}]K_A - K_A$-[Bound], the K_A and the B_{MAX} values can be obtained from the slope and the intercept of the plot [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 concentration of the unlabelled ligand under investigation with the aim of determining its IC_{50} value, 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 [22]; under controlled conditions $K_i = K_D = 1/K_A$.

2.2. Thermodynamic parameters determination

Measurements of K_A values at different temperatures allow the equilibrium thermodynamic parameters $\Delta G^\circ = -RT \ln K_A$ ($T = 298.15$ K) and ΔH° and ΔS° to be obtained. Two cases can be distinguished.

1. The standard specific heat difference of the equilibrium (ΔC_p°) is nearly zero. In this case the van't Hoff equation $\ln K_A = -\Delta H^\circ/RT + \Delta S^\circ/R$ gives a linear plot $\ln K_A$ versus $1/T$ and the standard enthalpy can be calculated from the slope, $-\Delta H^\circ/R$, and the standard entropy from the intercept, $\Delta S^\circ/R$, or as $(\Delta H^\circ - \Delta G^\circ)/T$, with $T = 298.15$ K and $R = 8.314$ J K^{-1} mol^{-1}.
2. ΔC_p° is different from zero. In this case the van't Hoff plot is often parabolic and other mathematical methods are available [21,23] for the analysis.

2.3. Linear regressions and t tests

All calculations were performed using the computer programs system Graph Pad Prism (Graph Pad, San Diego, CA, USA).

3. G-protein coupled receptors

Table 1 reports the thermodynamic data for the five GPCR which have so far been studied at a reasonable level of accuracy from a thermodynamic of view. The ranges of ΔG°, ΔH° and $-T\Delta S^\circ$ values of binding for both agonists and antagonists are given together with a qualitative classification of the prevailing equilibrium driving force (last column).

Only three out of the five GPCRs reported in Table 1 are actually discriminated. This is shown in Fig. 1 which summarises, in the form of $-T\Delta S^\circ$ versus ΔH° plots, the results of the thermodynamic measurements performed for each single receptor system.

As for the β-adrenergic receptor, agonists cluster in the exothermic region ($-79 \leq \Delta H^\circ \leq -17$ kJ mol^{-1}) with negative or small positive standard entropy values ($-8 \leq -T\Delta S^\circ \leq 44$ kJ mol^{-1}). Agonist binding is therefore classified as enthalpy-driven. Conversely the antagonist binding is mostly or totally entropy-driven ($-21 \leq \Delta H^\circ \leq 16$ kJ mol^{-1}; $-53 \leq -T\Delta S^\circ \leq -16$ kJ mol^{-1}).

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 G-protein coupled receptors so far studied ($T = 298.15$ K; EDF = equilibrium driving force)

Receptor	n	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$-T\Delta S°$ (kJ mol^{-1})	EDF
β-Adrenoceptors [8]					
Agonists	8	−39.3 to −25.9	−79 to −17	−8 to 44	H-driven
Antagonists	12	−52.3 to 31.2	−21 to 16	−53 to −16	S and H-driven
Adenosine A_1 [7,10]					
Agonists	14	−59.9 to −34.0	18 to 46	−106 to −61	S-driven
Antagonists	11	−49.2 to −24.4	−37 to −12	−18 to 6	H and S-driven
Adenosine A_{2A} [10]					
Agonists	7	−50.2 to −27.2	7 to 50	−83 to −53	S-driven
Antagonists	5	−35.0 to −26.2	−36 to −7	−28 to 10	H and S-driven
Dopamine D_2 [12]					
Agonists	4	−45.6 to −33.9	−68 to 3	−47 to 29	no discriminated
Antagonists	15	−57.3 to −24.0	−89 to 56	−105 to 44	no discriminated
Serotonin 5-HT_{1A} [14]					
Agonists	8	−58.1 to −35.8	−65 to 58	−109 to 20	no discriminated
Antagonists	7	−49.0 to −28.8	15 to 80	−109 to −47	no discriminated

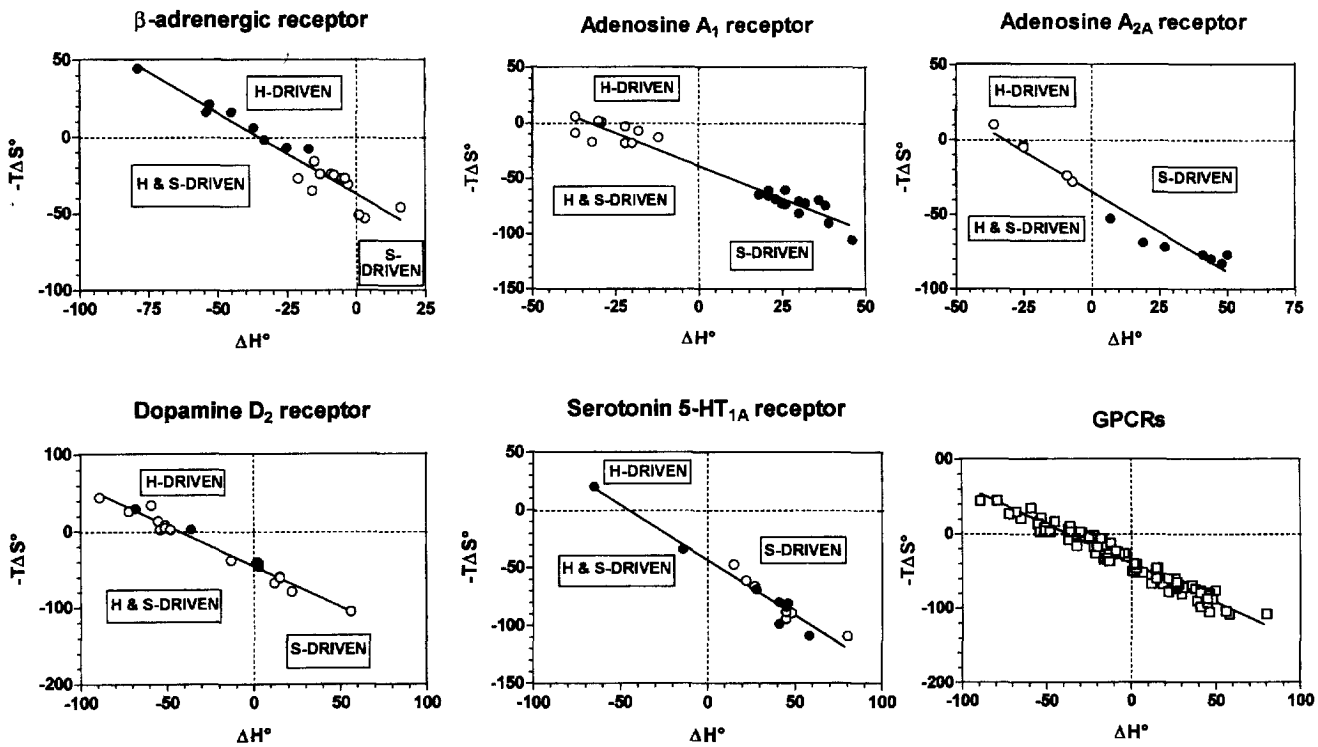

Fig. 1. $-T\Delta S°$ vs. $\Delta H°$ scatter plots of GPCRs, obtained from thermodynamic data of agonists (full circles) and antagonists (open circles) binding to the G-protein coupled receptors so far studied. All the values reported in a common scatter plot (open squares) lie on the same regression line (continuous line) of equation $T\Delta S°$ (kJ mol^{-1}) = 1.05(±0.02) $\Delta H°$ (kJ mol^{-1}) + 39.7(±0.9) ($n = 91$; $r = 0.975$). The slope and intercept values obtained for $-T\Delta S°$ vs. $\Delta H°$ scatter plots of the single receptor systems are not significantly different from the corresponding values of the common scatter plot (t test, $\alpha = 0.05$; $t = 0.49$, $P = 0.64$ for slope values; $t = 0.11$, $P = 0.92$ for intercept values).

Agonist binding at the adenosine A_1 receptor can be classified as totally entropy-driven ($18 \leq \Delta H° \leq 46$ kJ mol^{-1}; $-106 \leq -T\Delta S° \leq -61$ kJ mol^{-1}), while antagonist binding is mostly or totally enthalpy-driven ($-37 \leq \Delta H° \leq -12$ kJ mol^{-1}; $-18 \leq -T\Delta S° \leq 6$ kJ mol^{-1}). A similar result, from a qualitative point of view, has also been obtained for the adenosine A_{2A} receptor: the agonist binding is totally entropy-driven ($7 \leq \Delta H° \leq 50$ kJ mol^{-1}; $-83 \leq -T\Delta S° \leq 53$ kJ mol^{-1}) and the antagonist binding is essentially enthalpy-driven ($-36 \leq \Delta H° \leq -7$ kJ mol^{-1}; $-28 \leq -T\Delta S° \leq 10$ kJ mol^{-1}).

As far as the dopamine D_2 receptor is concerned, the antagonist binding can be totally enthalpy-driven, or enthalpy- and entropy-driven, or totally entropy-driven ($-89 \leq \Delta H° \leq 56$

kJ mol^{-1}; $-105 \leq -T\Delta S^{\circ} \leq 44$ kJ mol^{-1}). A similar behaviour has been found for the agonists ($-68 \leq \Delta H^{\circ} \leq 3$ kJ mol^{-1}; $-47 \leq -T\Delta S^{\circ} \leq 29$ kJ mol^{-1}). Agonists and antagonists do not show in this case the thermodynamic discrimination.

The antagonist binding at the 5-HT$_{1A}$ receptor is totally entropy-driven ($15 \leq \Delta H^{\circ} \leq 80$ kJ mol^{-1}; $-109 \leq -T\Delta S^{\circ} \leq -47$ kJ mol^{-1}), but the agonist binding can be totally enthalpy-driven, or enthalpy- and entropy-driven, or totally entropy-driven ($-65 \leq \Delta H^{\circ} \leq 58$ kJ mol^{-1}; $-109 \leq -T\Delta S^{\circ} \leq 20$ kJ mol^{-1}). Also in this case agonists and antagonists cannot be discriminated from a thermodynamic point of view.

An overall analysis of all the thermodynamic data reported in Table 1 indicates that the variability of ΔH° (-89 to 80 kJ mol^{-1}) and $-T\Delta S^{\circ}$ (-109 to 44 kJ mol^{-1}) values is much greater with respect to the variability of the ΔG° values (-58.1 to -24.0 kJ mol^{-1}).

The correlation equation of the GPCR data reported on a common $-T\Delta S^{\circ}$ versus ΔH° scatter plot (Fig. 1, GPCR) is:

$$T\Delta S^{\circ}\ (\text{kJ mol}^{-1}) = 39.7(\pm 0.9) + 1.05(\pm 0.02)\Delta H^{\circ}\ (\text{kJ mol}^{-1}) \quad (1)$$

$n=91$; $r=0.975$; $s=8.57$; $P<0.0001$)

The slope and intercept values of $-T\Delta S^{\circ}$ versus ΔH° plots of each single receptor system ($r \geq 0.96$; $P<0.0001$) have been analysed according to a t test. They are not significantly different ($\alpha=0.05$) from the corresponding values of Eq. (1) ($n=6, t=0.49, P=0.64$ for slope values; $n=6, t=0.11$, $P=0.92$ for intercept values). This result indicates that the ΔH° and $-T\Delta S^{\circ}$ data of the single GPCR systems are independently correlated on the same straight line.

4. Ligand-gated channel receptors

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

All the LGICR discriminate the agonists from the antagonists. This is shown in Fig. 2 which summarises in the form of $-T\Delta S^{\circ}$ versus ΔH° plots the results of the thermodynamic measurements performed for each single system.

Agonist binding to the nicotinic receptor is essentially enthalpy-driven ($-53 \leq \Delta H^{\circ} \leq -29$ kJ mol^{-1}; $-21 \leq -T\Delta S^{\circ} \leq 12$ kJ mol^{-1}), whereas antagonist binding is totally entropy-driven ($9 \leq \Delta H^{\circ} \leq 68$ kJ mol^{-1}; $-92 \leq -T\Delta S^{\circ} \leq -29$ kJ mol^{-1}).

As for the glycine receptor, the agonist binding is classified as entropy-driven ($2 \leq \Delta H^{\circ} \leq 20$ kJ mol^{-1}; $-56 \leq -T\Delta S^{\circ} \leq -24$ kJ mol^{-1}) and the antagonist binding mostly as enthalpy-driven ($-58 \leq \Delta H^{\circ} \leq -15$ kJ mol^{-1}; $-15 \leq -T\Delta S^{\circ} \leq 28$ kJ mol^{-1}).

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

Also for LGICR, an overall analysis of the thermodynamic data indicates that the variability of ΔH° (-58 to 68 kJ mol^{-1}) and $-T\Delta S^{\circ}$ (-95 to 60 kJ mol^{-1}) values is much greater with respect to the variability of ΔG° values (-52.9 to -20.7 kJ mol^{-1}). The correlation equation, reported on a common $-T\Delta S^{\circ}$ versus ΔH° scatter plot (Fig. 2, LGICR), is:

$$T\Delta S^{\circ}\ (\text{kJ mol}^{-1}) = 36(\pm 1) + 0.97(\pm 0.04)\Delta H^{\circ}\ (\text{kJ mol}^{-1}) \quad (2)$$

($n=44$; $r=0.953$; $s=9.18$; $P<0.0001$)

Table 2
Ranges of thermodynamic parameters, ΔG°, ΔH° and $-T\Delta S^{\circ}$, observed for the binding of agonists and antagonists to the four ligand-gated ion channel receptors so far studied ($T=298.15$ K; EDF = equilibrium driving force)

Receptor	n	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	$-T\Delta S^{\circ}$ (kJ mol^{-1})	EDF
Glycine [17]					
Agonists	4	−45.4 to −22.9	2 to 20	−56 to −24	H-driven
Antagonists	7	−43.3 to 23.1	−58 to −15	−15 to 28	H and S-driven
GABA$_A$ [18]					
Agonists	6	−38.5 to −28.4	−1 to 14	−48 to −28	S-driven
Antagonists	5	−46.4 to −29.7	−23 to −12	−31 to −14	H and S-driven
Serotonin 5-HT$_3$ [19]					
Agonists	7	−52.3 to −28.5	18 to 53	−95 to −60	S-driven
Antagonists	4	−52.9 to −37.2	−16 to 0	−53 to −21	H and S-driven
Nicotinic [21]					
Agonists	6	−50.7 to −34.3	−53 to −29	−21 to 12	H and S-driven
Antagonists	5	−36.2 to −20.7	9 to 68	−92 to −29	S-driven

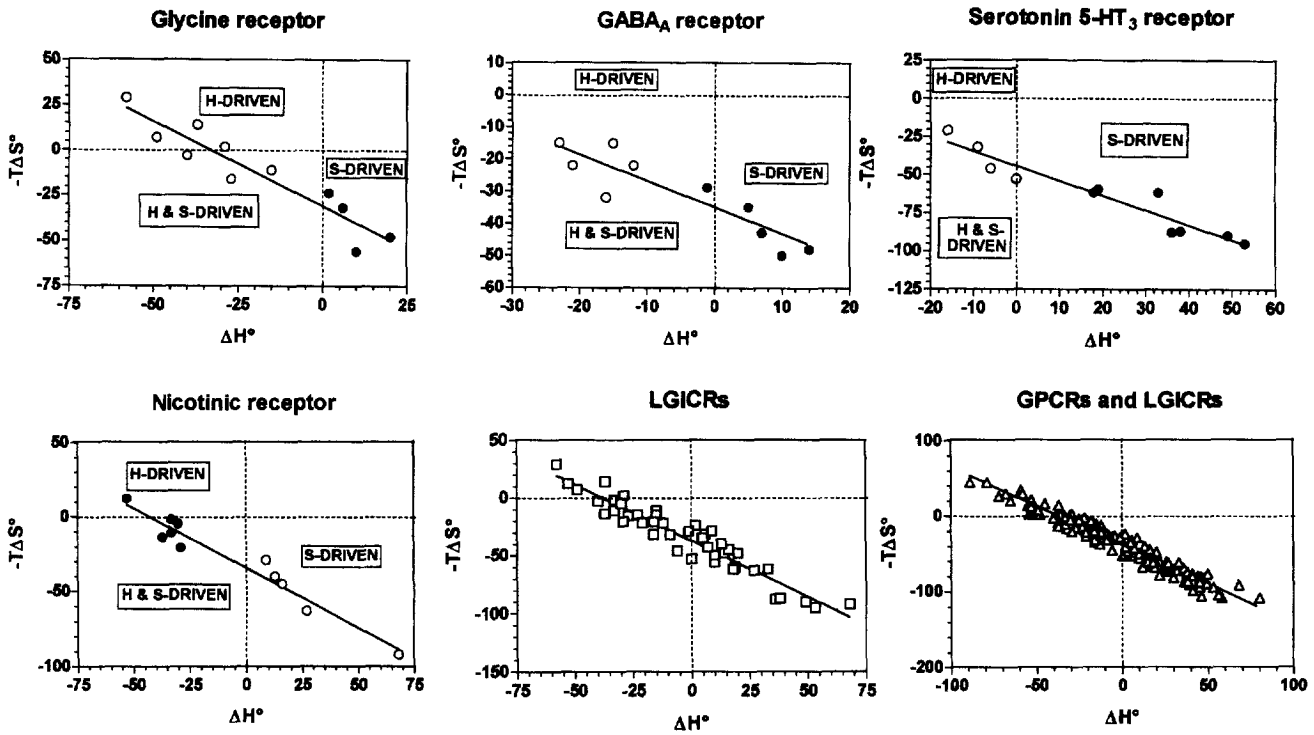

Fig. 2. $-T\Delta S^\circ$ vs. ΔH° scatter plots of LGICRs, obtained from thermodynamic data of agonists (full circles) and antagonists (open circles) binding to the ligand-gated ion channel receptors so far studied. All the values reported in a common scatter plot (open squares) lie on the same regression line (continuous line) of equation $T\Delta S^\circ$ (kJ mol^{-1}) = 0.97(±0.05) ΔH° (kJ mol^{-1}) + 36.8(±1.4) (n = 44; r = 0.953). The slope and intercept values obtained for $-T\Delta S^\circ$ vs. ΔH° scatter plots of the single LGICR systems are not significantly different from the corresponding values of the common scatter plot (t test, α = 0.05; t = 1.98, P = 0.12 for slope values; t = 0.23, P = 0.83 for intercept values). The $-T\Delta S^\circ$ vs. ΔH° scatter plot of the overall data of GPCRs and LGICRs (open triangles) gives a regression line of equation $T\Delta S^\circ$ (kJ mol^{-1}) = 1.04(±0.02) ΔH° (kJ mol^{-1}) + 38.8(±0.8) (n = 135; r = 0.971). The slope and intecept values are not significantly different from those of the equations of all the single receptor systems (t test, α = 0.05; t = 0.53, P = 0.64 for slope values; t = 0.11, P = 0.92 for intercept values).

The t test (α = 0.05) of slope and intercept values indicates that Eq. (2) does not differ significantly (n = 5, t = 1.99, P = 0.12 for slope values; n = 5, t = 0.23, P = 0.83 for intercept values) from the corresponding equations of the single LGICR systems ($r \geq 0.89$, $P \leq 0.0002$). Also in this case ΔH° and $-T\Delta S^\circ$ data of the single receptors appear independently correlated on the same straight line. Moreover Eqs. (1) and (2) are not statistically different. In fact a common $-T\Delta S^\circ$ versus ΔH° scatter plot of GPCR and LGICR (Fig. 2) data gives a regression line of equation

$$T\Delta S^\circ \text{ (kJ mol}^{-1}) = 38.8(\pm 0.8) + 1.04(\pm 0.02)\Delta H^\circ \text{ (kJ mol}^{-1}) \quad (3)$$

(n = 135; r = 0.971; s = 8.87; P < 0.0001)

Eq. (3) does not significantly differ from those obtained for all the single receptor systems reported in Figs. 1 and 2 (t test, α = 0.05, n = 10, t = 0.53, P = 0.64 for slope values; n = 10, t = 0.11, P = 0.92 for intercept values).

5. Discussion

Eq. (3) and the related t test are indicative of the presence of an enthalpy–entropy compensation phenomenon [24,25] for each single receptor system reported in Tables 1 and 2. This phenomenon seems to be a common feature in all cases of drug-receptor binding [6] and its general implications have been discussed by different authors [3,24,26,27]. Recently the enthalpy–entropy compensation phenomenon has been attributed, for drug–receptor interactions, to the solvent reorganisation that accompanies the receptor binding process in diluted solutions [27]. According to this point of view, while ΔG° values are most probably determined by the features of the ligand–receptor binding process, ΔH° and $-T\Delta S^\circ$ values appear strongly affected by the rearrangements occurring in the solvent [27].

What is not easy to understand is why agonists and antagonists may be discriminated in a thermodynamical sense (that is located in two disjointed regions of the E–E compensation band) if the compensation itself is mainly to be ascribed to a simple rearrangement of water molecules bearing little relationships with both binding affinity and intrinsic activity. This problem has already been debated for the β-adrenergic [8] and adenosine A_1 receptors [7], both belonging to the class of GPCRs, without finding a comprehensive explanation also because not all GPCRs are actually thermodynamically discriminated [12,14].

A new hypothesis can now be suggested in connection with LGIC receptors. Thermodynamic discrimination in

ligand-gated ion channels can be understood by admitting that the ΔH° (and compensation-related $-T\Delta S^{\circ}$) values are determined by both 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.

References

[1] G. Gilli, P.A. Borea, in: G.A. Jeffrey, J.F. Piniella (Eds.), The Application of Charge-density Research to Chemistry and Drug Design, Plenum, New York, 1991, pp. 241–286.
[2] D.B. Bylund, H.I. Yamamura, in: H.I. Yamamura, S.J. Enna, M.J. Kuhar (Eds.), Methods in Neurotransmitter Receptor Analysis, Raven Press, New York, 1990, pp. 1–36.
[3] B. Testa, P. Jenner, G.J. Kilpatrick, N. El Tayar, H. van der Waterbeemd, C.D. Marsden, Biochem. Pharmacol. 36 (1987) 4041–4046.
[4] R. Hitzemann, Trends Pharmacol. Sci. 9 (1988) 408–411.
[5] R.B. Raffa, F. Porreca, Life Sci. 44 (1989) 245–248.
[6] P. Gilli, V. Ferretti, P.A. Borea, G. Gilli, J. Phys. Chem. 98 (1994) 1515–1518.
[7] P.A. Borea, K. Varani, L. Guerra, P. Gilli, G. Gilli, Mol. Neuropharmacol. 2 (1992) 273–281.
[8] G.A. Weiland, K.P. Minneman, P.B. Molinoff, Nature 281 (1979) 114–117.
[9] G.A. Weiland, K.P. Minneman, P.B. Molinoff, Mol. Pharmacol., 18 (1980) 341–347.
[10] P.A. Borea, A. Dalpiaz, K. Varani, L. Guerra, G. Gilli, Biochem. Pharmacol. 49 (1995) 461–469.
[11] P.A. Borea, A. Dalpiaz, K. Varani, S. Gessi, G. Gilli, Life Sci. 59 (1996) 1373–1388.
[12] G.J. Kilpatrick, N. el Tayar, H. van der Waterbeemb, P. Jenner, B. Testa, C.D. Marsden, Mol. Pharmacol. 30 (1986) 226–234.
[13] E.P. Duarte, C.R. Oliveira, A.P. Carvalho, Eur. J. Pharmacol. 147 (1988) 227–239.
[14] A. Dalpiaz, P.A. Borea, S. Gessi, G. Gilli, Eur. J. Pharmacol. 312 (1996) 107–114.
[15] H. Möhler, G. Richards, Nature 294 (1981) 763–765.
[16] A. Doble, J. Neurochem. 40 (1983) 1605–1612.
[17] A.R. Gomez, M. Garcia Calvo, J. Vasquez, C.G. Marvizon, T. Valdiviero, F. Major Jr., J. Neurochem. 52 (1989) 1775–1780.
[18] C. Maksay, Mol. Pharmacol. 46 (1994) 336–340.
[19] P.A. Borea, A. Dalpiaz, S. Gessi, G. Gilli, Eur. J. Pharmacol. 298 (1996) 329–334.
[20] G. Maksay, J. Neurochem. 67 (1996) 407–412.
[21] P.A. Borea, K. Varani, P. Gilli, G. Gilli, Biochem. Pharmacol., in press.
[22] Y.C. Cheng, W.H. Prusoff, Biochem. Pharmacol. 22 (1973) 3099–3108.
[23] J.P. Osborne, J. Palumbo, H.B. Brewer, H. Edelhoch, Biochemistry 15 (1976) 317–320.
[24] E. Tomlinson, Int. J. Pharm. 13 (1983) 115–144.
[25] J.E. Leffler, E. Grunwald, Rates and Equilibria of Organic Reactions, Wiley, New York, 1963.
[26] R.R. Krug, W.G. Hunter, R.A. Grieger, Nature 261 (1976) 566–567.
[27] E. Grunwald, C. Steel, J. Am. Chem. Soc. 117 (1995) 5687–5692.