Chapter 8
Binding Thermodynamic Characteristics of Adenosine Receptor Ligands

Fabrizio Vincenzi, Katia Varani, and Pier Andrea Borea

Abstract Receptor binding thermodynamics is a powerful tool to gain deep insight, at the molecular level, of the events that occur during drug-receptor interactions. This chapter focuses on the determination of thermodynamic parameters based on the van't Hoff analysis as a traditional method to discover the enthalpic and entropic contributions during drug-receptor binding. Thermodynamic parameters of adenosine receptor ligands such as standard free energy (ΔG°), standard enthalpy (ΔH°), and standard entropy (ΔS°) are reported, discussed, and compared with those observed for other membrane receptors investigated from a thermodynamic point of view. The available thermodynamic data are evaluated in terms of two important physical phenomena, the thermodynamic discrimination and enthalpy-entropy compensation. Thermodynamic parameters obtained by means of radioligand binding studies for adenosine receptor ligands, as well as for other classes of receptors, represent relevant information to the drug design and optimization providing a benefit to the drug discovery process.

Keywords Thermodynamics · Enthalpy · Entropy · Free energy · van't Hoff equation · Adenosine receptors

8.1 Introduction

Thermodynamic analysis offers invaluable information on drug-receptor interactions potentially unavailable by other means. With regard to the binding of agonist or antagonists, drug-receptor interactions are usually characterized by a single measure of affinity that is quantified by the use of the equilibrium association constant (K_A) or, more commonly, its reciprocal the dissociation constant (K_D). The typical receptor binding assays performed at a single temperature provide little information about the molecular mechanisms underlying the interaction of a drug with a given receptor. In fact, the simple determination of a ligand affinity makes it possible to

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P. A. Borea et al. (eds.), *The Adenosine Receptors*, The Receptors 34,
https://doi.org/10.1007/978-3-319-90808-3_8

calculate the standard free energy ΔG° ($\Delta G^\circ = -RT\ln K_A$) but not its two components, the equilibrium standard enthalpy (ΔH°) and entropy (ΔS°), as defined by the Gibbs equation $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$. Standard enthalpy can be employed as a quantitative indicator of the changes in intermolecular bond energies which develop during binding (Borea et al. 2000; Holdgate and Ward 2005). Standard entropy can be considered an indicator of the rearrangements undergone by the solvent molecules during the same process (Gilli et al. 1994). Similar to most other biochemical reactions, the forces typically involved in drug-receptor interactions are not covalent, but rather a combination of non-covalent bonds such as hydrogen bonds, van der Waals forces, and hydrophobic interactions. When a drug molecule interacts with a receptor, it triggers a rearrangement of not only the receptor molecule with which it couples but also of the solvent molecules from which it uncouples.

The simultaneous optimization of enthalpy and entropy is complicated by several factors. First is the difficulty to optimize the forces that contribute to the binding enthalpy, and, second, the enthalpy gain is often compensated by an entropy loss. On the contrary, the binding entropy is easier to optimize because it is dependent primarily on the hydrophobic effect and is less affected by enthalpy compensation. Consequently, the recent trend has been toward increasingly hydrophobic, poorly soluble, entropically optimized drug candidates. However, it appears that a better binding enthalpy is critical for the development of improved drugs (Freire 2008). A favorable interaction enthalpy indicates that the drug establishes good and strong interactions with the target compensating the unfavorable enthalpy associated with desolvation. Conversely, an unfavorable binding enthalpy usually is an indication that polar groups are not forming strong bonds with the target and that the desolvation penalty dominates (Freire 2008). The most effective drug design and development platform comes from an integrated process. The understanding of the energetic basis of molecular interactions utilizing all available information from structural, thermodynamic, and biological studies is essential to realize an effective drug design (Garbett and Chaires 2012).

8.2 The van't Hoff Equation

The forces typically involved in drug-receptor interactions are not covalent, but rather are one or more of the following types: hydrogen bonds (of various strengths), van der Waals (and London) forces, hydrophobic interactions, and other similar phenomena. Because drug-receptor interactions are typically reversible, they are generally ascribable to standard equilibrium thermodynamic analysis. It is increasingly acknowledged that, to fully appreciate relevant molecular properties of potential drug candidates in a drug-design process, there is a need for thermodynamic studies. Traditionally, van't Hoff analysis has been used for thermodynamic studies.

There are two major ways of measuring thermodynamic parameters. One way has been proposed by the Dutch chemist J.H. van't Hoff in 1884. The van't Hoff equation provides information about the temperature dependence of the equilibrium constant. The van't Hoff equation may be derived from the Gibbs-Helmholtz equation, which gives the temperature dependence of the Gibbs free energy as

$$\Delta G^{\circ} = -RT \ln K_{\mathrm{A}}$$

(where T is the temperature in Kelvin and R is the ideal gas constant = 8.314 J/K/mol)

Because ΔG is related to the change in enthalpy (ΔH°) and entropy (ΔS°) by the equation $\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ}$, the former equation can be rearranged to

$$\ln\left(K_{\mathrm{A}}\right) = \left(-\Delta H^{\circ} / R\right)\left(1/T\right) + \Delta S^{\circ} / R$$

which is the integrated form of the van't Hoff equation. It actually follows from the van't Hoff equation $\mathrm{d}(\ln Keq)/\mathrm{dT} = \Delta H^{\circ}/RT^2$ and is an approximation that is valid when ΔH° and ΔS° are not temperature dependent. It is worth noting that this equation represents a linear relationship between $\ln(K_{\mathrm{A}})$ and $1/T$ with slope = $-\Delta H^{\circ}/R$ and y-intercept = $\Delta S^{\circ}/R$. It is a common practice in thermodynamic analysis of pharmacological interactions to determine K_{A} at several different temperatures and then construct a van't Hoff plot from which ΔH° and ΔS° are determined from the slope and the y-intercept of the resultant data plotted as $\ln(K_{\mathrm{A}})$ against $1/T$ (which is a line if the heat capacity is independent of temperature) (Fig. 8.1a). For an endothermic reaction, the slope is negative, and so as the temperature increases, the equilibrium constant increases, as shown in Fig. 8.1b. For an exothermic reaction, the slope is positive, and so as temperature increases, the equilibrium constant decreases, as illustrated in Fig. 8.1c.

The terms ΔG°, ΔH°, and ΔS° indicate the measurements made under standard state conditions of 1 atmosphere, unit activity (1 M concentration), and at 1 M hydrogen ion concentration (pH 0). A smaller error in ΔH° is obtained if ΔS° is determined first from the van't Hoff plot and then ΔH° from $\Delta H^{\circ} = \Delta G^{\circ} + T\Delta S^{\circ}$. Therefore, a method based on K_{D} measurements over a range of temperatures combined with van't Hoff plot analysis has been successfully applied to different receptor systems to obtain the thermodynamic terms of Gibbs equation (Borea et al. 2000).

Different receptorial systems have been so far studied in greater detail from a thermodynamic point of view, most of which concern membrane receptors: (i) G-protein-coupled receptors such as adenosine A_1 (Borea et al. 1994, 1996a, 1996b, Dalpiaz et al. 1998, 1999, 2000, 2002), A_{2A} (Borea et al. 1995; Baraldi et al. 1998), A_{2B} (Gessi et al. 2008), and A_3 (Merighi et al. 2002); β-adrenergic (Weiland et al. 1979; Contreras et al. 1986); dopamine D_2 (Kilpatrick et al. 1986; Agui et al. 1988; Duarte et al. 1988); and serotonin 5-HT_{1A} (Dalpiaz et al. 1995, 1996); (ii) ligand-gated ion channel

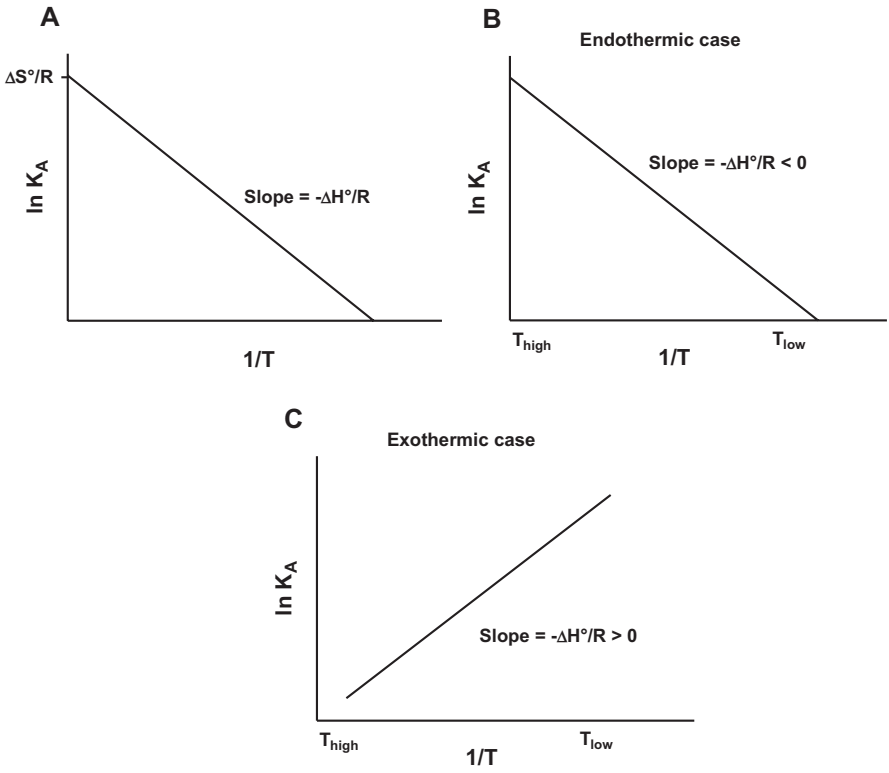

Fig. 8.1 The slope and intercept of a van't Hoff plot (**a**) and van't Hoff plot in endothermic (**b**) or exothermic (**c**) case

receptors such as glycine (Ruiz-Gómez et al. 1989), $GABA_A$ (Ruiz-Gómez et al. 1989), 5-HT_3 (Borea et al. 1996a; Maksay 1996), and nicotinic (Banerjee and Ganguly 1995, 1996; Borea et al. 1998, 2004); and (iii) the receptor for glucocorticoid hormones (Eliard and Rousseau 1984).

ΔG°, ΔH°, ΔS°, and $\Delta C_p{}^\circ$ (standard heat capacity) values have been collected for a remarkable number of ligands, including agonists, partial agonists, inverse agonists, or antagonists, both in the absence and in the presence of suitable modulators. The information provided by these data could be very useful from a pharmacological and pharmaceutical point of view, allowing us to discover new thermodynamic relationships related to drug−receptor interactions and their molecular mechanisms. As an example, ΔH° and ΔS° values can be used, in some membrane receptors, as indicators of the agonist or antagonist behavior of the ligands, the agonist and antagonist binding being, respectively, entropy-driven ($\Delta S^\circ \gg 0$; $\Delta H^\circ \geq 0$) and enthalpy-driven ($\Delta H^\circ \ll 0$; $\Delta S^\circ \leq 0$ or >0) or vice versa. This phenomenon, called thermodynamic discrimination, has been monitored for β-adrenergic, adenosine, glycine, $GABA_A$, serotonin 5-HT_3, and nicotinic membrane receptors.

Thermodynamic discrimination would hold even if the antagonists are to be classified in a different way, in agreement with the fact that a large number of antagonists of several membrane receptors have been recognized as inverse agonists, in touch with theoretical predictions indicating neutral antagonists as minority species in pharmacological space (Kenakin 2004). Another thermodynamic aspect, which characterizes all membrane receptors, is the ΔC_p° value nearly zero, a phenomenon which is not completely understood and is not usual in reactions involving biomacromolecules in solution (Sturtevant 1977).

8.3 Affinity Constant and Thermodynamic Parameters Determination

8.3.1 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 radiolabeled ligand. For a generic binding equilibrium

$$L + R \rightleftarrows LR$$

(where 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

$$[LR] / [L_{MAX} - LR] = [\text{Bound} / \text{Free}] = [B_{MAX}] K_A - K_A [\text{Bound}]$$

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 (Cheng and Prusoff 1973); under controlled conditions $K_i = K_D = 1/K_A$.

8.3.2 Thermodynamic Parameters Determination

Measurements of K_A values at different temperatures allow the equilibrium thermodynamic parameters $\Delta G° = -RT \ln K_A$ and $\Delta H°$ and $\Delta S°$ to be obtained.

Two cases can be distinguished:

(a) The standard specific heat difference of the equilibrium ($\Delta C_p°$) is nearly zero. In this case the van't Hoff equation $\ln K_A = -\Delta H°/RT + \Delta S°/R$ gives a linear plot $\ln K_A$ versus $1/T$ and 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}.

(b) $\Delta C_p°$ is different from zero. In this case the van't Hoff plot is often parabolic and other mathematical methods are available for the analysis (Borea et al. 2000).

8.4 Binding Thermodynamics of Adenosine Receptor Ligands

In the field of adenosine receptors, binding thermodynamic analysis has been performed at A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors and has added important findings such as the thermodynamic discrimination of agonists from antagonists and the recurrent phenomenon of entropy-enthalpy compensation (Gilli et al. 1994; Borea et al. 1994, 1995; Merighi et al. 2002; Gessi et al. 2008). All the examined compounds display essentially linear van't Hoff plots (Fig. 8.2). This behavior indicates that $\Delta Cp°$ (standard specific heat difference of the equilibrium) values of the drug-receptor binding equilibrium are nearly zero or in other words that $\Delta H°$ values are not significantly affected by temperature in the range investigated (0–30 °C). This phenomenon suggests that the conformational changes needed to produce the pharmacological effect are relatively small in this class of molecules most probably because larger modifications would make the association of the receptor with the cell membrane unstable. In addition, such linearity appears to be a typical property of the drug-membrane receptor binding at variance with the most binding processes between molecules and biomacromolecules occurring in solution (Sturtevant 1977; Tomlinson 1983; Grunwald and Steel 1995). Table 8.1 summarizes the thermodynamic parameters of adenosine receptor ligands where the ranges of $\Delta G°$, $\Delta H°$, and $\Delta S°$ for both agonist and antagonist binding ($n = 85$) are given together with a qualitative classification of the equilibrium driving force. Agonist binding at the A_1 adenosine receptors can be classified as totally entropy-driven ($9 \leq \Delta H° \leq 50$ kJ/mol; $-106 \leq -T\Delta S° \leq -61$ kJ/mol), while antagonist binding is enthalpy- and entropy-driven ($-44 \leq \Delta H° \leq -12$ kJ/mol; $-18 \leq -T\Delta S° \leq 7$ kJ/mol/K) (Borea et al. 1994; Lorenzen et al. 2000). As for the A_{2A} adenosine receptors, the agonist binding is totally entropy-driven ($7 \leq \Delta H° \leq 50$ kJ/mol; $-83 \leq -T\Delta S° \leq -53$ kJ/mol/K), and the antagonist is enthalpy- and entropy-driven ($-60 \leq \Delta H° \leq -7$ kJ/mol; $-28 \leq -T\Delta S° \leq 10$ kJ/mol/K) (Borea et al. 1995). In a similar way, agonists at

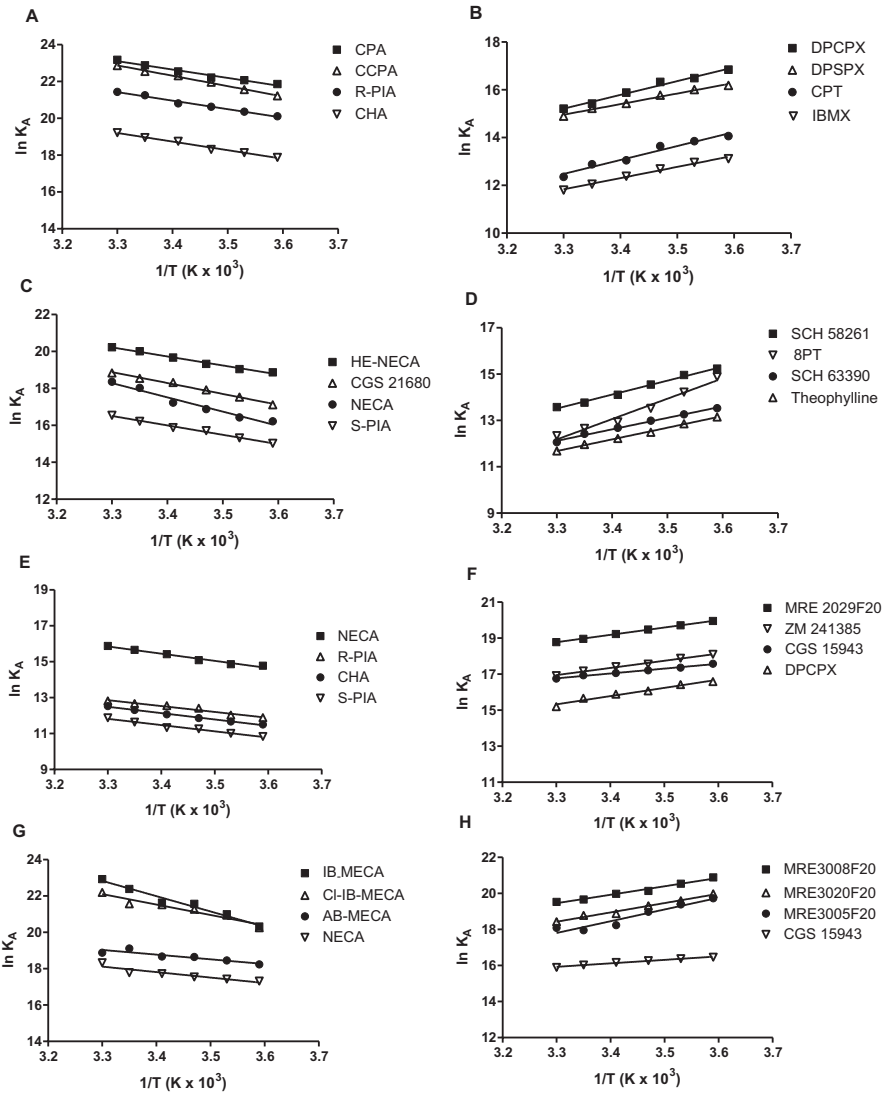

Fig. 8.2 Representative van't Hoff plots showing the effect of temperature on the equilibrium association constants of selected adenosine agonists or antagonists for A_1ARs (**a**, **b**), A_{2A}ARs (**c**, **d**), A_{2B}ARs (**e**, **f**), and A_3ARs (**g**, **h**)

A_{2B} adenosine receptors show a totally entropy-driven binding ($7 \leq \Delta H^\circ \leq 23$ kJ/mol; $-65 \leq -T\Delta S^\circ \leq -37$ kJ/mol), while antagonist binding is enthalpy- and entropy-driven ($-20 \leq \Delta H^\circ \leq -40$ kJ/mol; $-27 \leq -T\Delta S^\circ \leq -3$ kJ/mol) (Gessi et al. 2008). Similarly for A_3 adenosine receptors, the thermodynamic parameters fall in the ranges $21 \leq \Delta H^\circ \leq 67$ kJ/mol; $-122 \leq -T\Delta S^\circ \leq -67$ kJ/mol for agonists and $-52 \leq \Delta H^\circ \leq -9$ kJ/mol; $-24 \leq -T\Delta S^\circ \leq -5$ kJ/mol for antagonists showing that agonist binding is always totally entropy-driven while antagonist binding is

Table 8.1 Thermodynamic parameters, $\Delta G°$, $\Delta H°$, and $\Delta S°$, of a series of typical adenosine receptor ligands

Adenosine receptor subtypes	*N*	$\Delta G°$ kJ/mol	$\Delta H°$ kJ/mol	$\Delta S°$ J/mol/K	EDF	References
Adenosine A_1						Borea et al. (1994)
Agonists	23	–60 to –25	9 to 50	205 to 356	S-driven	
Antagonists	16	–49 to –24	–44 to –12	–23 to 60	H&S-driven	
Adenosine A_{2A}						Borea et al. (1995)
Agonists	7	–50 to –27	7 to 50	178 to 278	S-driven	
Antagonists	16	–50 to –26	–60 to –7	–34 to 94	H&S-driven	
Adenosine A_{2B}						Gessi et al. (2008)
Agonists	6	–43 to –29	7 to 23	123 to 219	S-driven	
Antagonists	6	–47 to 40	–40 to –20	10 to 91	H&S-driven	
Adenosine A_3						Merighi et al. (2002)
Agonists	6	–54 to –41	21 to 67	225 to 410	S-driven	
Antagonists	5	–49 to –33	–52 to –9	16 to 81	H&S-driven	

Note: Temperature used = 298.15 K; *N* = number of ligands; $\Delta G°$ = standard free energy; $\Delta H°$ = standard free enthalpy; $\Delta S°$ = standard free entropy; EDF = equilibrium driving force.

enthalpy- and entropy-driven (Varani et al. 2000; Merighi et al. 2002). The overall analysis of the thermodynamic data indicates that the variability of $\Delta H°$ (–224 to 90 kJ/mol) and $\Delta S°$ (–590 to 456 J/mol/K) values is again much greater than that of the $\Delta G°$ values (–63 to –24 kJ/mol), suggesting the possibility that enthalpy and entropy could be proposed as indicators of the pharmacological profile of adenosine ligands. In agreement with the idea that while $\Delta H°$ values are determined by the features of the ligand-receptor binding process, $\Delta S°$ values are determined by the rearrangements occurring during the binding in the solvent-drug and solvent-receptor interfaces. As a matter of fact, in the adenosine agonist-receptor interaction, the insertion of the ribose moiety and the depletion of the water network induce conformation changes in the receptor site able to mediate the final biological effect. Consequently, a high degree of correlation between intrinsic activity and $\Delta S°$ values was reported for adenosine ligands acting as full or partial agonists and as antagonists (Borea et al. 1994).

As for all adenosine receptor subtypes, it appears clearly apparent the thermodynamic interdependence of $\Delta H°$ and $-T\Delta S°$ where all the experimental points appear to be arranged along the same diagonal line, according to the equation: $\Delta H°$ (kJ/mol) = –41 (±2) + 288 (±3) $\Delta S°$ kJ/mol/K ($n = 85$, $r = 0.981$, $p < 0.001$) (Fig. 8.3).

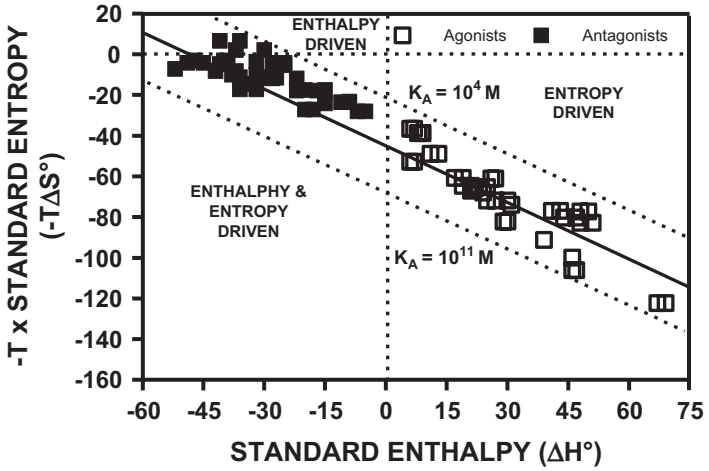

Fig. 8.3 Scatter plot of $-T\Delta S°$ against $\Delta H°$ for adenosine compounds. Full and open symbols indicate antagonists and agonists, respectively. All points lie on the same regression line. The two dashed lines indicate the loci of the points representing possible combinations of $\Delta H°$ and $-T\Delta S°$ values giving rise to the two different equilibrium constants indicated ($K_A = 10^4$ M^{-1} and $K_A = 10^{11}$ M^{-1})

8.5 Binding Thermodynamics of G-Protein-Coupled Receptor Ligands

Table 8.2 and Fig. 8.4a summarize the thermodynamic parameters of G-protein-coupled receptors (GPCRs) so far studied where the ranges of $\Delta G°$, $\Delta H°$, and $\Delta S°$ for both agonist and antagonist binding ($n = 203$) are given together with a qualitative classification of the equilibrium driving force. The analysis of the data revealed that six out of the ten GPCRs reported are discriminated. For dopamine D_2 receptor ligands, thermodynamic values for antagonist ($-89 \leq \Delta H° \leq 59$ kJ/mol; $-105 \leq -T\Delta S° \leq 107$ kJ/mol/K) and agonist binding ($-224 \leq \Delta H° \leq 90$ kJ/mol; $-136 \leq -T\Delta S° \leq 176$ kJ/mol/K) are scattered over their complete range. Therefore, agonists and antagonists do not show thermodynamic discrimination (Duarte et al. 1988). A similar behavior is shown by the 5-HT_{1A} receptors where antagonist ($15 \leq \Delta H° \leq 80$ kJ/mol; $-109 \leq -T\Delta S° \leq -47$ kJ/mol/K) and agonist binding ($-65 \leq \Delta H° \leq 58$ kJ/mol; $-109 \leq -T\Delta S° \leq 20$ kJ/mol/K) do not suggest any agonist-antagonist discrimination (Dalpiaz et al. 1996). As for opioid receptors, antagonists ($-52 \leq \Delta H° \leq 5$ kJ/mol; $-15 \leq -T\Delta S° \leq -2$ kJ/mol/K) and agonists ($-42 \leq \Delta H° \leq 12$ kJ/mol; $-19 \leq -T\Delta S° \leq -4$ kJ/mol/K) are not thermodynamically discriminated (Borea et al. 1988; Li et al. 1998). This result is in qualitative agreement with that reported for the binding of nociceptin receptors where the agonist binding was entropy-driven (Varani et al. 1998). The cholecystokinin CCK_2 receptor ligands have been also investigated to verify the discrimination of agonists and antagonists. The finding of a lack of thermodynamic discrimination between

Table 8.2 Thermodynamic parameters, $\Delta G°$, $\Delta H°$, and $\Delta S°$, of different G-protein-coupled receptor ligands

GPCRs	*N*	$\Delta G°$ kJ/mol	$\Delta H°$ kJ/mol	$\Delta S°$ J/mol/K	EDF	References
Dopamine D_2						Duarte et al. (1988)
Agonists	11	−53 to −34	−224 to 90	−590 to 456	ND	
Antagonists	22	−59 to −24	−89 to 59	−359 to 352	ND	
Serotonin 5-HT_{1A}						Dalpiaz et al. (1996)
Agonists	8	−58 to −36	−65 to 58	−67 to 366	ND	
Antagonists	7	−49 to −29	15 to 80	158 to 366	ND	
Opioid						Borea et al. (1988)
Agonists	9	−63 to −47	−42 to 12	13 to 64	ND	
Antagonists	6	−59 to-50	−52 to 5	5 to 49	ND	
Cholecystokinin CCK_2						Harper et al. (2007a)
Agonists	2	−47 to −49	−71 to −64	−74 to −58	ND	
Antagonists	6	−51 to −36	−65 to −3.5	−67 to 152	ND	
β-Adrenoceptors						Weiland et al. (1979)
Agonists	14	−51 to −26	−143 to −17	−312 to 27	H-driven	
Antagonists	23	−61 to −31	−21 to 16	54 to 178	H&S-driven	
Histamine H_3						Harper et al. (2007b)
Agonists	7	−58 to −48	−31 to −23	198 to 311	S-driven	
Antagonists	3	−55 to −47	6 to 45	57 to 120	H&S-driven	
Cannabinoid CB_1						Merighi et al. (2010)
Agonists	5	−51 to −36	17 to 59	213 to 361	S-driven	
Antagonists	3	−49 to −33	−52 to −26	−12 to 38	H&S-driven	
Cannabinoid CB_2						Merighi et al. (2010)
Agonists	5	−48 to −40	27 to 48	234 to 300	S-driven	
Antagonists	3	−41 to −32	−19 to −17	43 to 74	H&S-driven	

Note: Temperature used = 298.15 K; *N* = number of ligands; $\Delta G°$ = standard free energy; $\Delta H°$ = standard free enthalpy; $\Delta S°$ = standard free entropy; EDF = equilibrium driving force.

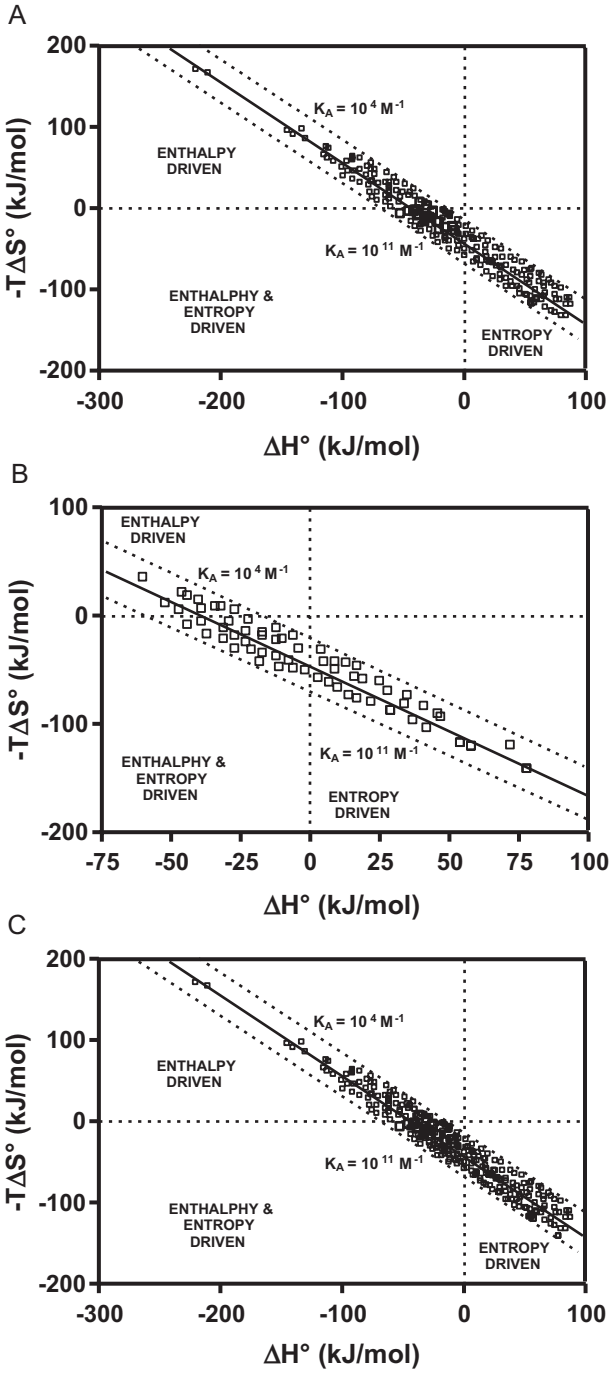

Fig. 8.4 Scatter plot of $-T\Delta S°$ versus $\Delta H°$ values for the GPCR (**a**, $n = 203$), LGICR (**b**, $n = 68$), and GPCR and LGICR (**c**, $n = 271$) agonists and antagonists. All points lie on the same regression line. The two dashed lines indicate the loci of the points representing possible combinations of $\Delta H°$ and $-T\Delta S°$ values giving rise to the two different equilibrium constants indicated ($K_A = 10^4\ M^{-1}$ and $K_A = 10^{11}\ M^{-1}$)

agonists and antagonists at the CCK_2 receptors has been explained by suggesting that small molecules may each have a unique combination of individual interactions with the receptors (Harper et al. 2007a, 2008). As for the β-adrenergic receptor, agonist cluster is in the exothermic region ($-143 \leq \Delta H^\circ \leq -17$ kJ/mol) with negative or weakly positive standard entropy values ($-8 \leq -T\Delta S^\circ \leq 93$ kJ/mol/K). Agonist binding has therefore to be classified as enthalpy-driven. Conversely, the antagonist binding is mostly or totally entropy-driven ($-21 \leq \Delta H^\circ \leq 16$ kJ/mol; $-53 \leq -T\Delta S^\circ \leq -16$ kJ/mol/K) (Weiland et al. 1979). The thermodynamic parameters for CB1 receptors fall in the ranges $17 \leq \Delta H^\circ \leq 59$ kJ/mol and $213 \leq \Delta S^\circ \leq 361$ kJ/mol for agonists and $-52 \leq \Delta H^\circ \leq -26$ kJ/mol and $-12 \leq \Delta S^\circ \leq 38$ kJ/mol for antagonists. The thermodynamic parameters for CB2 receptors fall in the ranges $27 \leq \Delta H^\circ \leq 48$ kJ/mol and $234 \leq \Delta S^\circ \leq 300$ kJ/mol for agonists and $-19 \leq \Delta H^\circ \leq -17$ kJ/mol and $43 \leq \Delta S^\circ \leq 74$ kJ/mol for antagonists. Collectively, these data show that agonist binding is always totally entropy-driven while antagonist binding is enthalpy- and entropy-driven, indicating that CB1 and CB2 receptors are thermodynamically discriminated (Merighi et al. 2010). Finally, the finding that histamine H_3-receptor agonist binding was entropy-driven was explained by the disorganization of a solvation sphere around the ligands as they bind to the receptor (Harper et al. 2007b; Harper and Black 2007). Another possible explanation suggested was that the agonist binding at histamine H_3-receptors induces ternary complex formation and this brings to the large increase in entropy. Interestingly, the presence of salts such as $CaCl_2$ in the buffer solution changes the thermodynamic behavior of histamine ligands. In these experimental conditions, agonists and antagonists showed similar thermodynamic parameters. This may be a consequence of the capability of buffer salts to increase the hydration of the ligands so that more water has to be removed during the receptor binding interaction (Harper and Black 2007).

8.6 Binding Thermodynamics of Ligand-Gated Ion Channel Receptor Ligands

Analysis of thermodynamic parameters of ligand-gated ion channel receptor ligands (LGICR) has revealed that five out of six receptors are thermodynamically discriminated (Table 8.3, Fig. 8.4b). As for the glycine receptor, the agonist binding has to be classified as entropy-driven ($2 \leq \Delta H^\circ \leq 20$ kJ/mol; $-56 \leq -T\Delta S^\circ \leq -25$ kJ/mol), whereas the antagonist binding is mostly enthalpy-driven ($-58 \leq \Delta H^\circ \leq -15$ kJ/mol; $-15 \leq -T\Delta S^\circ \leq 29$ kJ/mol) (Ruiz-Gómez et al. 1989). Agonist binding to the $GABA_A$ receptor is entropy-driven ($-1 \leq \Delta H^\circ \leq 14$ kJ/mol; $-48 \leq -T\Delta S^\circ \leq -28$ kJ/mol), while antagonist binding is enthalpy- and entropy-driven ($-23 \leq \Delta H^\circ \leq -12$ kJ/mol; $-31 \leq -T\Delta S^\circ \leq -15$ kJ/mol) (Maksay 1994). A similar result is also obtained for the serotonin 5-HT_3 receptor where the agonist binding is totally entropy-driven ($18 \leq \Delta H^\circ \leq 53$ kJ/mol; $-95 \leq -T\Delta S^\circ \leq -60$ kJ/mol) and antagonist binding is both enthalpy- and entropy-driven ($-16 \leq \Delta H^\circ \leq 0$ kJ/mol; $-53 \leq -T\Delta S^\circ \leq -21$ kJ/mol)

Table 8.3 Thermodynamic parameters, $\Delta G°$, $\Delta H°$, and $\Delta S°$, of different ligand-gated ion channel receptor ligands

LGICRs	N	$\Delta G°$ kJ/mol	$\Delta H°$ kJ/mol	$\Delta S°$ J/mol/K	EDF	References
Glycine						Ruiz-Gómez et al. (1989)
Agonists	4	−48 to −24	2–20	94 to 188	S-driven	
Antagonists	7	−44 to −23	−58 to −15	−45 to 97	H&S-driven	
$GABA_A$						Maksay (1994)
Agonists	6	−40 to −30	−1 to 14	94 to 161	S-driven	
Antagonists	5	−48 to −30	−23 to −12	50 to 104	H&S-driven	
Serotonin $5\text{-}HT_3$						Borea et al. (1996a)
Agonists	7	−52 to −28	18 to 53	201 to 319	S-driven	
Antagonists	4	−53 to-37	−16 to 0	70 to 178	H&S-driven	
Nicotinic						Borea et al. (2004)
Agonists	7	−51 to −25	−58 to −29	−114 to 70	H&S-driven	
Antagonists	6	−37 to −21	9 to 82	97 to 409	S-driven	
$P2X_3$ purinergic						Varani et al. (2008)
Agonists	5	−46 to −41	−26 to −18	59 to 73	H&S-driven	
Antagonists	6	−40 to −30	14 to 36	149 to 249	S-driven	
$P2X_1$ purinergic						Varani et al. (2008)
Agonists	5	−46 to −37	−31 to −23	41 to 50	ND	
Antagonists	6	−30 to −25	−22 to −19	17 to 34	ND	

Note: Temperature used = 298.15 K; N = number of ligands; $\Delta G°$ = standard free energy; $\Delta H°$ = standard free enthalpy; $\Delta S°$ = standard free entropy; EDF = equilibrium driving force.

(Borea et al. 1996a). At variance with the other ion channel receptors, agonist binding to the nicotinic receptor is essentially enthalpy-driven ($-58 \leq \Delta H° \leq -29$ kJ/mol; $-21 \leq -T\Delta S° \leq 34$ kJ/mol), whereas antagonist binding is totally entropy-driven ($9 \leq \Delta H° \leq 82$ kJ/mol; $-122 \leq -T\Delta S° \leq -29$ kJ/mol) (Borea et al. 1998, 2004). $P2X_1$ and $P2X_3$ purinergic receptors have been also characterized from a thermodynamic point of view with the following parameters: $-31 \leq \Delta H° \leq -19$ kJ/

mol; $-15 \leq -T\Delta S^\circ \leq -5$ kJ/mol and $-26 \leq \Delta H^\circ \leq 36$ kJ/mol; $-74 \leq -T\Delta S^\circ \leq -18$ kJ/mol, respectively. Interestingly, $P2X_1$ and $P2X_3$ purinergic receptors have a different thermodynamic behavior as demonstrated by the fact that agonists and antagonists for $P2X_1$ receptors show similar enthalpy and entropy values. On the contrary $P2X_3$ receptors can be considered thermodynamically discriminated because agonist binding is enthalpy- and entropy-driven and antagonist binding is totally entropy-driven (Varani et al. 2008). The overall $-T\Delta S^\circ$ versus ΔH° scatter plot of the data for GPCRs and LGICRs is reported in Fig. 8.4c.

8.7 Conclusions

The adenosine receptor ligand so far investigated displays essentially linear van't Hoff plots indicating that ΔCp° (standard specific heat difference of the equilibrium) values of the drug-receptor binding equilibrium are nearly zero or in other words that ΔH° values are not significantly affected by temperature in the range investigated (0–30 °C). This phenomenon seems to indicate that the conformational changes needed to produce the pharmacological effect are relatively small in this class of molecules most probably because larger modifications would make the association of the receptor with the cell membrane unstable. In addition, such linearity appears to be a typical property of the drug-membrane receptor binding at variance with the most binding processes between molecules and biomacromolecules occurring in solution (Sturtevant 1977; Tomlinson 1983; Grunwald and Steel 1995). As for all adenosine receptor subtypes, it appears clearly apparent the thermodynamic interdependence of ΔH° and $-T\Delta S^\circ$ where all the experimental points appear to be arranged along the same diagonal line, according to the equation:

$$\Delta H^\circ\left(\text{kJ/mol}\right) = -41\left(\pm 2\right) + 288\left(\pm 3\right)\Delta S^\circ \text{kJ/mol/K}\left(n = 85,,r = 0.981,,p < 0.001\right)$$

For the overall GPCR agonists and antagonists investigated, the equation was

$$\Delta H^\circ\left(\text{kJ/mol}\right) = -41\left(\pm 2\right) + 304\left(\pm 4\right)\Delta S^\circ \text{kJ/mol/K}\left(n = 203,,r = 0.975,,p < 0.001\right)$$

while for the 68 LGICR ligands was

$$\Delta H^\circ\left(\text{kJ/mol}\right) = -37\left(\pm 2\right) + 250\left(\pm 3\right)\Delta S^\circ \text{kJ/mol/K}\left(n = 68,,r = 0.965,,p < 0.001\right)$$

The regression equation obtained by plotting standard enthalpy and entropy data of 271 ligands performed on 16 different membrane receptor systems belonging to the GPCR and LGICR families was

$$\Delta H^{\circ}\left(\text{kJ / mol}\right) = -41\left(\pm 2\right) + 297\left(\pm 3\right)\Delta S^{\circ}\text{kJ / mol / K}\left(n = 271,,r = 0.971,,p < 0.001\right)$$

These equations could be rewritten as $\Delta H° = \beta \Delta S°$, which is the form for a case of enthalpy-entropy compensation with a compensation temperature of 302 K. It is generally accepted that entropy and enthalpy values in a scatter plot are arranged on the same diagonal band encompassed between the two dashed lines which represent the loci points defined by the limiting K_D values of 100 μM and 10 pM. This phenomenon seems to be a common feature in all cases of drug-receptor binding. The enthalpy-entropy compensation phenomenon has been attributed for drug-receptor interactions to the solvent reorganization that accompanies the receptor binding process in diluted solutions (Tomlinson 1983; Grunwald and Steel 1995). According to this point of view, while the features of the ligand-receptor binding process most probably determine $\Delta H°$ values, $\Delta S°$ values appear strongly affected by the rearrangements occurring in the solvent. It seems reasonable to assume that solvent effects might be responsible for the in vitro thermodynamic discrimination between agonists and antagonists observed for the majority of LGICRs and some of the GPCRs studied. The finding that the binding of adenosine receptor agonists is entropy-driven can be explained by the disorganization of a solvation area around the ligand-receptor interaction. Another possible explanation is that the agonists induce a change in receptor conformation perhaps into a less-constrained state, which, in turn, leads to the formation of a ternary complex with a G-protein, and this consequently results in a decrease in the solvation of the cytosolic side of the receptor. The finding of the increase in enthalpy associated with antagonist binding may be explained by hydrogen bond formation and van der Waals interactions occurring between the ligands and the binding pocket which cannot be compensated for by changes in entropy that result from agonist-induced conformational changes in the receptor.

In conclusion, the thermodynamic data represent relevant information to the drug design and development (Holdgate and Ward 2005). In particular, when compounds have similar affinities, their enthalpy values can be used to select one as the preferred lead compound for optimization. A favorable enthalpy values implies better complementarity of the binding interfaces because enthalpy corresponds to the energy associated with the net change in non-covalent bonds. The knowledge of the thermodynamic parameters could help the discovery and characterization of novel selective receptor agonists or antagonists.

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