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Thermodynamics of 5-HT_3 receptor binding discriminates agonistic from antagonistic behaviour

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Abstract

Thermodynamic parameters $\Delta G°$, $\Delta H°$ and $\Delta S°$ of the binding equilibrium of eleven ligands (seven agonists and four antagonists) to the serotonin 5-HT_3 receptor subtype have been determined by affinity measurements carried out on rat cortex membranes at six different temperatures (0, 10, 20, 25, 30, 35°C) and van't Hoff plots. Affinity constants were obtained from saturation experiments of [^{3}H]*endo-N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1-methyl-1-*H*-indazole-3-carboxamide ([^{3}H]BRL 43694, a selective 5-HT_3 ligand) or by its displacement in inhibition assays for the other compounds. Van't Hoff plots were essentially linear in the temperature range investigated, showing that the $\Delta C_p°$ of the binding equilibrium is nearly zero. Thermodynamic parameters are in the range $18 \leq \Delta H° \leq 53$ kJ mol^{-1} and $202 \leq \Delta S° \leq 320$ J K^{-1} mol^{-1} for agonists and $-16 \leq \Delta H° \leq 0$ kJ mol^{-1} and $70 \leq \Delta S° \leq 179$ J K^{-1} mol^{-1} for antagonists indicating that agonistic binding is totally *entropy-driven* while antagonistic binding is relatively less *entropy-* and more *enthalpy-driven* in the $-T\Delta S°$ *versus* $\Delta H°$ plot the thermodynamic data are clearly arranged in separate clusters for agonists and antagonists, which, therefore, turn out to be *thermodynamically discriminated.* Experimental results are discussed according to the following main points: (i) the approximate linearity of the $\Delta H°$ *versus* $\Delta S°$ plot in terms of *enthalpy-entropy* compensation and (ii) the fact that $\Delta C_p° \simeq 0$ for practically all membrane receptors at variance with most reactions involving biomacromolecules in solution. Finally, the phenomenon of thermodynamical discrimination is reviewed and found to occur in five distinct membrane receptorial systems.

Keywords: 5-HT_3 receptor; Binding thermodynamics; Agonist and antagonist discrimination; Thermodynamic compensation

1. Introduction

5-Hydroxytryptamine (5-HT_3, serotonin) is an endogenous substance known to produce vasoconstriction (Rocha e Silva et al., 1953; Gaddum and Hameed, 1954) and peripheral nervous system stimulation (Gyermek and Bindler, 1962; Fozard et al., 1977), and to affect various functions of the central nervous system (Zifa and Fillion, 1992). The receptors reputed to mediate these effects have been intensively studied using both in vivo and in vitro pharmacological methods and radioligand binding. Three main classes have been distinguished by Bradley et al. (1986): 5-HT_1, 5-HT_2 and 5-HT_3, although more recent molecular biology studies have identified not less than seven main classes of putative 5-HT receptors (Boess and Martin, 1994; Hoyer et al., 1994).

From a pharmacological point of view, 5-HT_1 receptors are characterized by their high affinity for serotonin and 5-carboxamidotryptamine and are blocked by methiothepin and methysergide. They have been further subdivided (Peroutka, 1988) into two subtypes (5-HT_{1A} and 5-HT_{1B}) on the grounds of their spiperone and 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) specific binding features. It has been recognised that 5-HT_1 receptors are associated with the modulation of adenylyl cyclase activity (Zifa and Fillion, 1992; Boess and Martin, 1994).

5-HT_2 receptors display lower affinity for serotonin and much higher affinity for spiperone, ketanserin and mianserin. Three subtypes have been proposed, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, but only the first can be well characterised by its pharmacological profile, the other two sub-

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types having been identified because of the differences in their primary sequences (Boess and Martin, 1994). Serotonin binding to 5-HT_{2A} receptors is known to produce phosphatidyl-inositol hydrolysis (Zifa and Fillion, 1992; Boess and Martin, 1994).

5-HT_3 receptors, which are antagonized by cocaine, MDL 72222 or ICS 205-930 (tropisetron), are ligand-gated ion-channels, affecting the permeability to Na^+, K^+, Ca^{2+} ions (Peters et al., 1992).

We have recently undertaken a systematic investigation on the thermodynamic aspects of the binding equilibrium of serotoninergic drugs to the different 5-HT receptor subtypes aimed at having a direct knowledge of the enthalpic ($\Delta H°$) and entropic ($\Delta S°$) contributions to the equilibrium standard free energy ($\Delta G° = \Delta H° - T\Delta S° = -RT \ln K_A$; K_A = association constant). The present paper deals, in particular, with the binding thermodynamics of seven agonists and four antagonists at the rat cortex 5-HT_3 receptor. It will be shown that thermodynamic properties can discriminate between agonists and antagonists (in the sense that agonistic binding is much more entropy-driven) so that binding experiments in vitro are found to give information not only on ligand affinities but also on the nature of the pharmacological response. This effect, which has been called *thermodynamic discrimination* (Borea et al., 1992) was firstly observed by Weiland et al. (1979) for the β-adrenoceptor and only recently has been confirmed in other receptorial systems, i.e. adenosine A_1 and A_{2a} (Borea et al., 1992, 1995) and $GABA_A$ receptors (Maksay, 1994).

2. Materials and methods

2.1. Materials

[^{3}H]*endo-N*-(9-Methyl-9-azabicyclo[3.3.1]non-3-yl)-1-methyl-1-*H*-indazole-3-carboxamide ([^{3}H]BRL 43694, specific activity = 84.3 Ci/mmol) and Aquassure were obtained from NEN Research Products, Du Pont de Nemours Italiana, Milano, Italy. Serotonin, 1-(3-chlorophenyl)piperazine (*m*-CPP), 2-(1-piperazinil)quinoline (quipazine), *N*-(3-trifluoromethyl-phenyl)piperazine (TFMPP), *N*-phenylimido-carbonimidic-diamide (1-phenylbiguanide), 2-methyl-5-hydroxytriptamine (2-methyl-serotonin), 5-methoxytryptamine (mexamine), 1-methyl-*N*-(8-methyl-8-azabicyclo[3,2,1]oct-3-yl)-1*H*-indazole-3-carboxamide (LY-278.584), 1,2,3,4,10,14b-hexahydro-2-methyl dibenzo[*c*,*f*]-pyryzino[1,2*a*]azepine (mianserin) and 4-amino-5-chloro-*N*-[(2-diethylamino)ethyl]-2-methoxybenzamide (metoclopramide) were purchased from Research Biochemical International (RBI), Amersham Italia, Milan, Italy. Male Wistar rats were acquired from Nossan Laboratories (Varese, Italy). Unless otherwise stated, other materials were from standard sources.

2.2. Membrane preparation

Male Wistar rats (150–200 g) were decapitated and the whole brain (minus brainstem, striatum and cerebellum) was dissected on ice and disrupted in a Polytron (setting 5) in 10 volumes of 50 mM Hepes, pH 7.5. The homogenate was centrifuged at $50\,000 \times g$ for 10 min and the pellet was twice resuspended in the same buffer and centrifuged. After the last centrifugation the pellets were stored at −70°C. Prior to freezing, an aliquot of homogenate was removed for protein assays (Lowry et al., 1951).

2.3. Receptor binding assays

Binding assays were performed at 0, 10, 20, 25, 30 and 35°C essentially according to Nelson and Thomas (1989). Displacement experiments from 5-HT_3 receptors were performed in 2 ml of 50 mM Hepes, pH 7.5 containing 0.6 nM [^{3}H]BRL 43694 and membranes from 30 mg (wet weight) of tissue. Non-specific binding was defined as the binding in the presence of an excess of serotonin (10 μM); this is always $\leq 25\%$ of the total binding. Saturation experiments were carried out using at least eight concentrations of [^{3}H]BRL 43694 ranging from 0.1 to 5 nM. The incubation time ranged from 120 min at 0°C to 30 min at 35°C according to previous time course experiments. All buffer solutions were adjusted to maintain a constant pH of 7.5 at any desired temperature.

To determine IC_{50} values (where IC_{50} is the inhibitor concentration displacing 50% of the labelled ligand) solutions of the endogenous ligand were added in triplicate to the binding assay samples at a minimum of six different concentrations.

Separation of bound from free radioligand was performed by a rapid filtration though Whatman GF/B filters which were washed 3 times with ice cold buffer. Filter-bound radioactivity was measured by scintillation spectrometry after the addition of 4 ml of Aquassure. K_i values were calculated from the Cheng and Prusoff equation (Cheng and Prusoff, 1973). All binding data were analysed using the non-linear regression curve fitting computer program LIGAND (Munson and Rodbard, 1990).

2.4. Calculations

For a generic binding equilibrium L + R = LR (L = Ligand, R = Receptor), the affinity constant is 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. As $[LR]/[L_{MAX} - LR] = [Bound]/[Free] = [B_{MAX}]K_A - K_A[Bound]$, the K_A and B_{MAX} values can be obtained from the slope and intercept of the Scatchard plot [Bound]/[Free] versus [Bound]. In inhibition experiments, K_i values were calculated from the IC_{50} values according to the Cheng and Prusoff Eq. (17): $K_i = IC_{50}/(1 + [C^*]/K_D^*)$ where $[C^*]$ is the concentration of the radioligand and K_D its dissocia-

tion constant. The standard free energy is calculated as $\Delta G^\circ = -RT\ln K_A$ at 298.15 K, and the standard enthalpy, ΔH°, from the ΔG° measurements at different temperatures. For this purpose two general cases can be distinguished.

(1) The difference of standard specific heats at constant pressure of the equilibrium (ΔC_p°) is nearly zero: in this case the equation $(\delta \ln K_A/\delta(1/T) = -\Delta H^\circ/R$ gives a linear van't Hoff plot ln K_A versus $(1/T)$, and the standard enthalpy can be calculated from its slope, $-\Delta H^\circ/R$; the standard entropy is calculated as $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$ 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 plot ΔG° versus T is often parabolic and other mathematical methods (Osborne et al., 1976) for calculating the thermodynamic parameters of the equilibrium are available.

In the present case the van't Hoff plots can be considered to be essentially linear (see below) and the first method was applied.

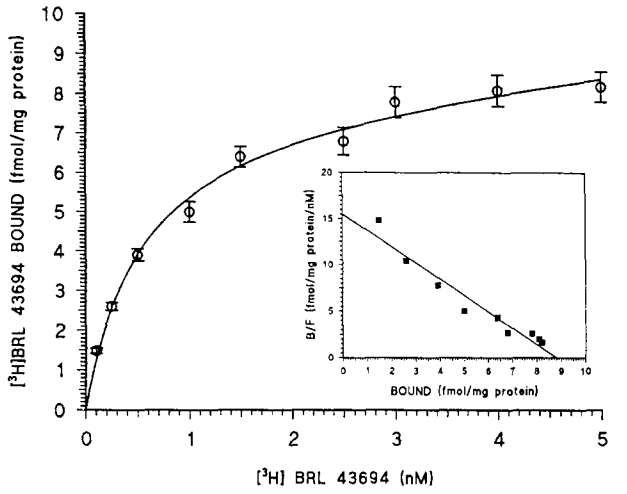

Fig. 1. Saturation curve and its Scatchard plot (inset) obtained at 25°C for the binding equilibrium of [^{3}H]BRL 43694 to rat cortex membranes. The linarity of the Scatchard plot indicates the presence of a single class of high affinity binding sites.

3. Results

Table 1 shows the values of inhibitory binding constants, K_i, measured, at the six chosen temperatures and for the selected eleven ligands, by a displacement of [^{3}H]BRL 43694 from rat cortex 5-HT$_3$ receptors; K_D and B_{MAX} values derived from saturation experiments of [^{3}H]BRL 43694 are also shown. Because of the general equivalence between K_i values and dissociation equilibrium constants, the K_D values measured for [^{3}H]BRL 43694 can be considered to be homogenous with the other tabulated data.

Fig. 1 illustrates the saturation curve (and its Scatchard plot in the inset) obtained at 25°C for the binding equilibrium of [^{3}H]BRL 43694. The Schatchard plot is essentially linear and computer analysis of the data (Munson and Rodbard, 1990) failed to show a significantly better fit to a two-site than to a one-site binding model, indicating that only one class of high affinity binding sites is present under our experimental conditions. Similar conclusions can be drawn from the analysis of the displacement curves concerning all other compounds in Table 1.

The temperature dependence of the affinity constants, $K_A = 1/K_i$, is shown in the van't Hoff plots, ln K_A versus $1/T$, of Figs. 2 and 3, which report the results for the seven agonists and the four antagonists investigated, respectively. Van't Hoff plots appear to be linear for all compounds in the complete range of temperature (0–35°C). Affinity constant of serotonin displays a sudden drop at

Table 1
Equilibrium binding parameters at six different temperatures expressed as: (i) dissociation constants, K_D (nM) and B_{MAX} (fmol/mg protein) for compound 1. ([^{3}H]BRL 43694) derived from saturation experiments to rat cortex 5-HT$_3$ receptors; (ii) inhibitory constants, K_i (nM), for compounds 2–11 obtained by displacement of 0.6 nM [^{3}H]BRL 43694 from the same receptors

Ligand		T (K)											
		273		283		293		298		303		308	
(a) Antagonists													
1. [^{3}H]BRL 43694	K_D =	0.52	(±0.03)	0.42	(±0.02)	0.54	(±0.03)	0.53	(±0.04)	0.41	(±0.03)	0.51	(±0.02)
	B_{MAX} =	8.1	(±0.4)	8.9	(±0.5)	9.5	(±0.6)	8.8	(±0.5)	9.1	(±0.6)	8.9	(±0.5)
2. Metoclopramide		163	(±9)	173	(±9)	227	(±12)	273	(±14)	296	(±15)	364	(±18)
3. Mianserin		40.2	(±2.7)	41.0	(±2.9)	47.7	(±2.3)	50.1	(±2.8)	59.4	(±3.0)	63.2	(±3.1)
4. LY-278.584		0.55	(±0.02)	0.60	(±0.03)	0.61	(±0.03)	0.64	(±0.03)	0.73	(±0.04)	0.77	(±0.04)
(b) Agonists													
5. Serotonin		91.2	(±6.4)	84.2	(±5.0)	59.3	(±4.1)	45.3	(±2.8)	45.1	(±2.7)	81.2	(±6.5)
6. *m*-CPP		27.3	(±1.6)	25.2	(±1.3)	18.2	(±0.9)	13.6	(±0.7)	12.7	(±0.6)	11.0	(±0.6)
7. Mexamine		31810	(±1900)	22730	(±1200)	12720	(±700)	9120	(±500)	8180	(±450)	6360	(±340)
8. Quipazine		1.9	(±0.1)	1.8	(±0.1)	0.91	(±0.06)	0.55	(±0.03)	0.41	(±0.02)	0.41	(±0.02)
9. TFMPP		3180	(±180)	2273	(±114)	1100	(±62)	1100	(±59)	770	(±41)	450	(±29)
10. 2-Methylserotonin		318	(±18)	273	(±15)	136	(±8)	59.1	(±3.2)	50.2	(±3.0)	31.4	(±2.2)
11. 1-Phenylbiguanide		200	(±12)	90	(±4)	54.3	(±3.1)	46.4	(±2.3)	17.3	(±1.0)	13.6	(±0.7)

Values are means of at least four experiments. Estimated standard deviations are in parentheses.

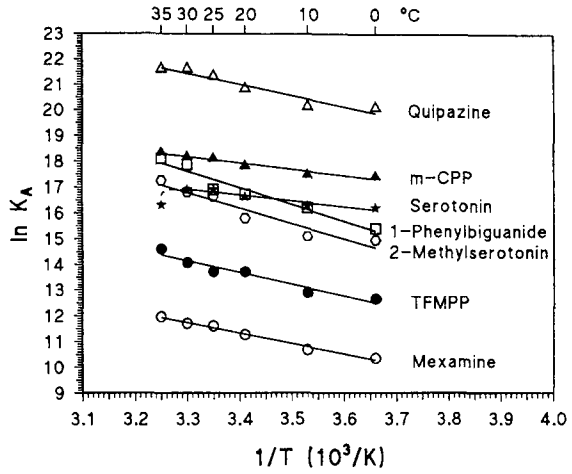

Fig. 2. Van't Hoff plots showing the effect of temperature on the equilibrium binding association constants, K_A, for the seven 5-HT_3 agonists investigated. All plots are fairly linear ($r \geq 0.95$) in the full temperature range from 0 to 35°C (0 to 30°C for serotonin).

35°C. Since a parabolic fit of K_i between 0–35°C is not statistically significant, only the linear portion between 0 and 30°C has been used. Slopes of van't Hoff plots are weakly positive for antagonists (practically zero for BRL 43694) but negative for agonists (Figs. 2 and 3).

Final thermodynamic parameters are reported in Table 2. $\Delta G°$ values range from −52.3 to −28.4 kJ mol^{-1} for agonists and −52.9 to −37.2 kJ mol^{-1} for antagonists. Equilibrium standard enthalpy, $\Delta H°$, and entropy, $\Delta S°$, values indicate that the binding of agonists is totally entropy-driven; the large increase in entropy ($202 \leq \Delta S° \leq 320$ J K^{-1} mol^{-1}), besides driving the binding, compensates for the endothermicity of the reaction ($18 \leq \Delta H° \leq 53$ kJ mol^{-1}). For antagonists $\Delta S°$ is always positive and $\Delta H°$ zero or weakly negative, so that the binding can be classified as entropy and enthalpy-driven with entropic

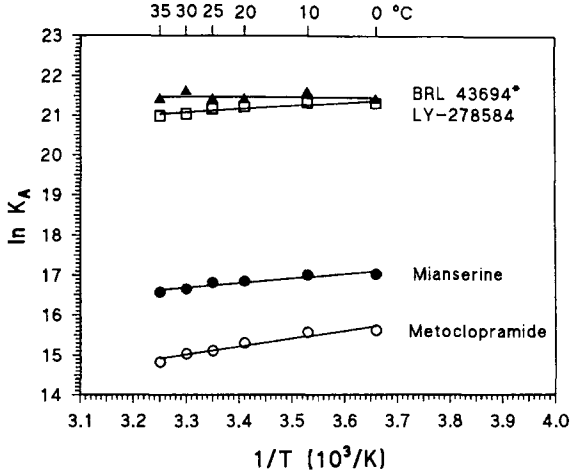

Fig. 3. Van't Hoff plots showing the effect of temperature on the equilibrium binding association constants, K_A, for the four 5-HT_3 antagonists investigated. All plots are essentially linear ($r \geq 0.92$) in the temperature range from 0 to 35°C.

Table 2
Thermodynamic parameters for the binding equilibrium of agonists and antagonists to serotonin 5-HT_3 receptors

Ligand	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})
(a) Antagonists			
1. [^{3}H]BRL 43694	−52.9 (±0.3)	0.21 (±3)	179 (± 9)
2. Metoclopramide	−37.2 (±0.2)	−16 (±2)	70 (± 7)
3. Mianserin	−41.3 (±0.2)	−9 (±2)	107 (± 6)
4. LY-278.584	−52.1 (±0.3)	−6 (±1)	154 (± 4)
(b) Agonists			
5. Serotonin	−41.4 (±0.2)	19 (±4)	202 (±14)
6. *m*-CPP	−44.5 (±0.3)	18 (±3)	209 (±11)
7. Mexamine	−28.4 (±0.1)	33 (±2)	207 (± 7)
8. Quipazine	−52.3 (±0.3)	36 (±5)	296 (±19)
9. TFMPP	−49.0 (±0.3)	38 (±5)	242 (±17)
10. 2-Methylserotonin	−40.6 (±0.3)	49 (±6)	301 (±21)
11. 1-Phenylbiguanide	−42.6 (±0.2)	53 (±6)	320 (±22)

$\Delta G°$, $\Delta H°$ and $\Delta S°$ values are given at 298.15 K. Estimated standard deviations are in parentheses.

prevalence ($-16 \leq \Delta H° \leq 0$ kJ mol^{-1}; $70 \leq \Delta S° \leq 179$ J K^{-1} mol^{-1}).

Standard deviations have been estimated to be in the range of 1–6 kJ mol^{-1} for $\Delta H°$, and 4–22 J K^{-1} mol^{-1} for $\Delta S°$.

4. Discussion

Fig. 4 summarizes in the form of a $-T\Delta S°$ versus $\Delta H°$ plot the results of the thermodynamic measurements given

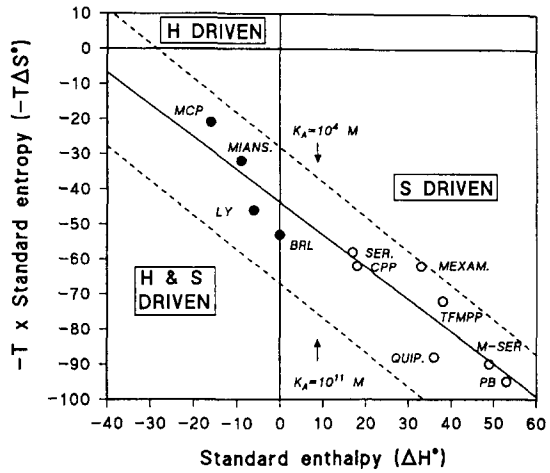

Fig. 4. $-T\Delta S°$ versus $\Delta H°$ (kJ mol^{-1}; $T = 298.15$K) scatter plot for 5-HT_3 receptor agonists (open circles) and antagonists (full circles). All points lie on a same regression line (continuous line) of equation $T\Delta S° = 43(\pm 3) + 0.9(\pm 0.1)\Delta H°$ ($n = 11$, $R = -0.950$). The two dashed lines indicate the *loci* of the points representing possible combinations of $\Delta H°$ and $-T\Delta S°$ values which give rise to two different association constants ($K_A = 10^4$ M and 10^{11} M). MCP = metoclopramide, MIANS. = Mianserin, LY = LY-278.584, BRL = BRL 43694, SER. = serotonin, CPP = *m*-CPP, Mexam. = mexamine, M-SER. = 2-methylserotonine, QUIP. = quipazine, PB = 1-phenylbiguanide.

in Table 2. The plot clearly shows that agonists cluster in a region of endothermicity and large entropy increase, while antagonists gather together in a different cluster of weak exothermicity and less favoured entropy change. It should be noted, however, that the binding of all ligands is, at least in part, entropy driven ($-95 \leq -T\Delta S^{\circ} \leq -21$ kJ mol^{-1}). This could be accounted for in terms of the disorganization of a large solvation sphere of the cationic ligand in view of the fact that all compounds studied are almost completely protonated at the assay pH of 7.5 (approximate pK_a range of 9–12 (Smith and Arthur, 1975)).

Experimental points of Fig. 4 are nearly arranged on a same line with equation:

$$T\Delta S^{\circ}\,(\text{kJ/mol at } 298.15\,\text{K}) = 43(\pm 3) + 0.9(\pm 0.1)\,\Delta H^{\circ}(\text{kJ/mol})$$

$$(n = 11, r = -0.95, s = 7.82, P < 0.01)$$

Parameters of this equation are similar to those observed for the binding of a variety of drugs to other receptors. For example, the regression slope and intercept are 1.14(±0.06) and 37(±2), respectively, for the binding of 24 different ligands to both adenosine A_1 and A_{2A} receptors (Borea et al., 1995). Such a linearity of the $-T\Delta S^{\circ}$ versus ΔH° plot is to be considered a particular case of the enthalpy-entropy compensation effect ($\Delta H^{\circ} = \beta\Delta S^{\circ}$; β = compensation temperature), a phenomenon which has been observed to hold, in general, for the binding of all ligands to all known membrane receptors, as recently discussed in some detail by different authors (Gilli et al., 1994; Testa et al., 1987).

The linearity of the van't Hoff plots (Figs. 2 and 3) deserves some comment. It indicates that the ΔC_p° for the binding equilibrium is nearly zero in the system studied, i.e. that the value of ΔH° is not significantly affected by temperature variations in the range investigated. The sudden affinity constant decrease found for serotonin at 35°C (see above) is a phenomenon which has been already observed in other cases and seems to occur more frequently for endothermic than exothermic drug-receptor interactions and for agonist than antagonist binding (Borea et al., 1988, 1992, 1995; Eliard and Rousseau, 1984). It suggests that, in vitro, the denaturation of receptors dissolved in disrupted membranes may start, after interaction with some particular ligands, at temperatures lower than those that are physiological for mammals.

The linearity of van't Hoff plots is not very common in reactions involving biomacromolecules in solution. It is known (Sturtevant, 1977) that the binding of coenzymes or substrates to enzymes, the unfolding of proteins, or the helix-coil transition in polynucleotides often occur with relevant changes of ΔC_p°, which are usually interpreted as due to modifications of the macromolecule surface exposed to solvent (Edsall and Gutfreund, 1983). The fact that this ΔC_p° change does not occur for membrane receptors (Gilli et al., 1994) can be considered as an indication that the conformational changes needed to produce the pharmacological effect are relatively small in this class of macromolecules, most probably because larger modifications would render a stable association of the receptor with the cell membrane problematic.

An interesting aspect of this research concerns the thermodynamical discrimination between agonists and antagonists which are found to cluster into two distinct regions of the $-T\Delta S^{\circ}$ versus ΔH° plot (Fig. 4). This phenomenon is not common to all membrane receptors and it seems proven, at least within the limits of the present experimentation, that it does not occur in the binding of agonists and antagonists to the dopamine D_2 (Zahniser and Molinoff, 1983; Kilpatrick et al., 1986; Duarte et al., 1988), benzodiazepine (Möhler and Richards, 1981;

Table 3
Average thermodynamical parameters ΔG°, ΔH° and $-T\Delta S^{\circ}$ (with standard deviations in parentheses) of agonists and antagonists for the receptorial systems for which the phenomenon of thermodynamic discrimination has so far been observed (n = number of drugs in the sample; EDF = equilibrium driving force; H = enthalpy; S = entropy)

Receptor	n	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	$-T\Delta S^{\circ}$ (kJ mol^{-1})	EDF
β-Adrenoceptor (Weiland et al., 1979)					
Agonists	8	−31.9 (±4.0)	−43 (±20)	+11 (±17)	*H*-driven
Antagonists	12	−39.3 (±9.0)	−6 (±10)	−32 (±12)	*S&H*-driven
Adenosine A_1 (Borea et al., 1992)					
Agonists	14	−44.4 (±7.2)	29 (± 8)	−74 (±12)	*S*-driven
Antagonists	11	−32.8 (±8.8)	−26 (± 8)	−7 (± 9)	*H&S*-driven
Adenosine A_{2a} (Borea et al., 1995)					
Agonists	7	−39.2 (±8.0)	34 (±16)	−70 (±16)	*S*-driven
Antagonists	5	−30.7 (±3.3)	−20 (±12)	−10 (±15)	*H&S*-driven
$GABA_A$ (Maksay, 1994)					
Agonists	6	−33.7 (±4.5)	7 (± 5)	−39 (± 8)	*S*-driven
Antagonists	5	−38.5 (±6.9)	−17 (± 4)	−20 (± 7)	*S&H*-driven
5-HT_3 (present work)					
Agonists	7	−42.7 (±7.6)	35 (±13)	−76 (±15)	*S*-driven
Antagonists	4	−45.9 (±7.8)	−8 (± 7)	−38 (±15)	*S&H*-driven

Kochman and Hirsch, 1982) and acetylcholine-nicotinic (Maelicke et al., 1977) receptors. Table 3 summarizes the thermodynamic data for the five receptors for which such discrimination has been so far observed. Average ΔG°, ΔH° and $-T\Delta S^\circ$ for both agonists and antagonists are given together with a qualitative classification of the prevailing equilibrium driving force (last column of Table 3). At the beginning of the eighties only one receptorial system displaying such a discrimination was known (Weiland et al., 1979), i.e. the β-adrenoceptor system for which agonistic and antagonistic binding is, respectively, enthalpy-driven, and entropy- and enthalpy-driven. According to their findings the authors (Weiland et al., 1979, 1980) suggested the hypothesis, based on information theory, that enthalpy-driven agonistic receptor binding mirrors the conformational changes which lead to the biological response. Successively several authors (Borea et al., 1995; Testa et al., 1987; Hitzemann, 1988; Gilli and Borea, 1991) supported a different interpretation of the reaction driving force in terms of the physico-chemical properties of drugs and receptor sites. Data of Table 3 show now that the activation message leading to the biological response can be transmitted by ligands which bind for purely entropic reasons. The thermodynamic compensation phenomenon can explain such an apparent contradiction indicating a strict connection between ΔH° and ΔS° in the binding processes, independently of the transduction system.

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