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Binding thermodynamics of 5-HT_{1A} receptor ligands

Alessandro Dalpiaz [b], Pier Andrea Borea [a,*], Stefania Gessi [a], Gastone Gilli [b]

[a] *Istituto di Farmacologia, Università di Ferrara, 44100 Ferrara, Italy*
[b] *Dipartimento di Chimica, Università di Ferrara, 44100 Ferrara, Italy*

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Abstract

The thermodynamic parameters $\Delta G°$, $\Delta H°$ and $\Delta S°$ of the binding equilibrium of 15 ligands (eight agonists and seven antagonists) to the 5-HT_{1A} receptor subtype have been determined by affinity measurements carried out on rat cortex membranes (minus striatum) at six different temperatures (0, 10, 20, 25, 30, 35°C), and by van't Hoff plots. Most of the compounds studied are tryptamine, phenylpiperazine and tetralin derivatives. Affinity constants were measured by saturation experiments for the selective 5-HT_{1A} receptor agonist [^{3}H]8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin ([^{3}H]8-OH-DPAT) and by inhibition assays of [^{3}H]8-OH-DPAT binding for the other compounds. Scatchard plots were monophasic in the full range of temperatures, indicating a single class of high affinity binding sites. Van't Hoff plots of all ligands were linear in the temperature range investigated (0–30°C or 0–35°C). 5-Hydroxytryptamine (serotonin) and 5-methoxy-tryptamine (mexamine) displayed a positive slope. Experimental data indicate that for 5-HT_{1A} receptor subtype agonists and antagonists are not thermodynamically discriminated. The results are discussed from a quantitative point of view with the aim of obtaining new details on the nature of the forces driving the 5-HT_{1A} binding at a molecular level.

Keywords: Binding thermodynamics; 5-HT_{1A} receptor; Drug-receptor interaction

1. Introduction

Thermodynamic parameters of the binding equilibrium of drugs to their receptors have aroused increasing interest in recent years due to the information they may provide on molecular binding mechanisms. In fact current receptor binding assays, performed at a single temperature, allow the determination of drug-receptor association constants, K_A, which give quantitative information on the ability of a drug to interact with a given receptor, but provide little information on the mechanism, at molecular level, underlying the interaction itself. Affinity constants, K_A, give the values of the standard free energy of the equilibrium, $\Delta G° = -RT \ln K_A$ which consists of two different terms according to the Gibbs equation $\Delta G° = \Delta H° - T\Delta S°$, $\Delta H°$ and $\Delta S°$ being the equilibrium standard enthalpy and entropy, respectively. These terms permit to distinguish the factors responsible for the drug receptor recognition phenomenon: the enthalpic term is a measure of the non-bonded interactions, as hydrogen bonding and multipolar or dispersion interactions, while the entropic term is a measure of the solvent reorganization. As a consequence thermodynamic data allow to evaluate if a drug interacts with a receptor by weak intermolecular bonds (which develop heat and are responsible for negative values of the Gibbs equation enthalpic term, $\Delta H°$) and/or by the rearrangement of the solvent molecules around the drug and receptor binding site, a phenomenon which contributes to enhance the energy distribution disorder of the system and is responsible for a positive value of the Gibbs equation entropic term, $\Delta S°$.

We have recently undertaken an analysis of the binding thermodynamic aspects of agonists and antagonists at serotonin receptors. According to the results obtained it was possible to suggest that the different equilibrium thermodynamic profile of serotonin (5-hydroxytryptamine) to 5-HT_{1A}, 5-HT_3 and 5-HT_{2A} receptor subtypes could be explained in terms of an identified amino acid difference in the receptor sequences between 5-HT_{2A} and the other two receptors (Dalpiaz et al., 1995); moreover it has been established that the binding of agonists and antagonists at the 5-HT_3 receptor is thermodynamically discriminated (Borea et al., 1996). The present paper deals with the binding thermodynamics of eight agonists and seven antag-

* Corresponding author. Istituto di Farmacologia, Via Fossato di Mortara 19, 44100 Ferrara, Italy. Tel.: Italy-532-291214; fax: Italy-532-291205.

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onists at the rat cortex 5-HT_{1A} receptors which are characterized, from a pharmacological point of view, by a selective sensitivity to the agonist 8-OH-DPAT (8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin, Bradley et al., 1986). It is generally accepted that 5-HT_{1A} receptors are involved in psychiatric disorders like depression and anxiety (Zifa and Fillion, 1992) modulating the intracellular levels of the second messenger c-AMP (Boess and Martin, 1994; Hoyer et al., 1994).

Most of the compounds studied are 3-(2-aminoethyl)indole (tryptamine), phenylpiperazine and tetralin derivatives (Fig. 1) belonging to chemical classes of compounds which can display high 5-HT_{1A} affinity and selectivity. All these compounds are described as 5-HT receptor agonists with the exception of the antagonist NAN-190; six more antagonists have therefore been added (Fig. 2) and three of these are also β-adrenoceptor antagonists (*S*-propranolol, *S*-pindolol and alprenolol). It will be shown that the thermodynamical data collected reveal new details concerning the nature of the mechanisms governing 5-HT_{1A} binding at a molecular level and that the binding thermodynamic profiles of *S*-propranolol, *S*-pindolol and alprenol are qualitatively the same at both 5-HT_{1A} receptors and β-adrenoceptors.

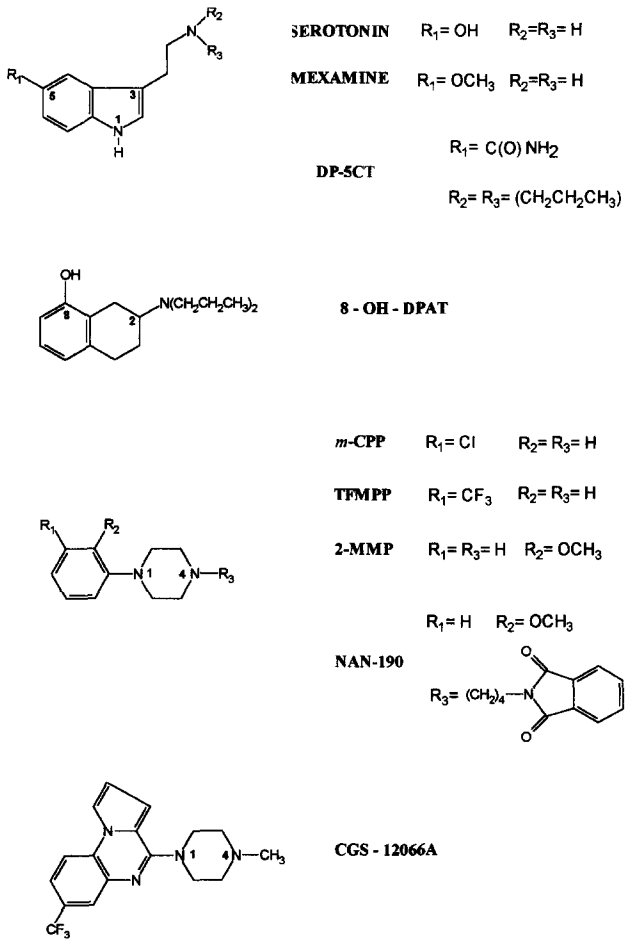

Fig. 1. Chemical formulas of the eight 5-HT_{1A} receptor agonists studied. The formula of the antagonist NAN 190 is also included.

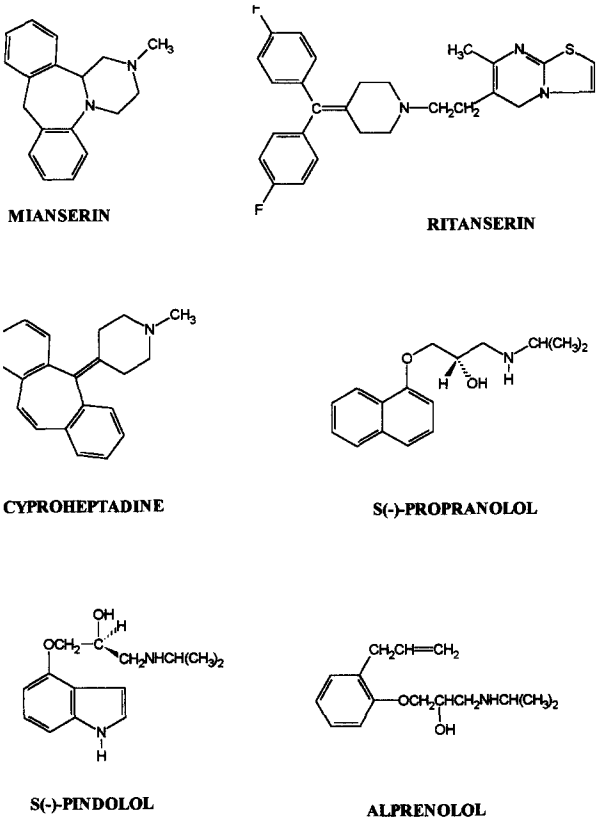

Fig. 2. Chemical formulas of the 5-HT_{1A} receptor antagonists studied (NAN-190 excluded).

2. Materials and methods

2.1. Materials

[^{3}H]8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin ([^{3}H]8-OH-DPAT, specific activity = 147.2 Ci/mmol) and Aquassure were obtained from NEN Research Products, Du Pont de Nemours Italiana, Milano, Italy. 5-Hydroxytryptamine (serotonin), 8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin (8-OH-DPAT), 5-methoxy-tryptamine (mexamine), *N*,*N*-dipropyl-5-carboxamidotryptamine (DP-5CT), *N*-(3-trifluoromethyl-phenyl)piperazine (TFMPP), 1-(2-methoxy-phenyl)piperazine (2-MPP), 1-(3-chlorophenyl)piperazine (*m*-CPP), 7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline (CGS-12066B), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190), 1,2,3,4,10,14b-hexahydro-2-methyl-dibenzo-[*c*,*f*]-pyryzino-[1,2*a*]azepine (mianserin), 4-(5*H*-dibenzo-[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine (cyproheptadine), 6-[2-[4-[*bis*(4-fluorophenyl)methylene]-1-piperidinyl]-ethyl]-7-methyl-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (ritanserin), 1[(1-methyl-ethyl)amino]-3-[2-(2-pro-

penyl)phenoxy]-2-propanol (alprenolol), 1-[(1-methylethyl)amino]-3-(1-naphtalenyloxy)-2-propanol (propranolol) and 1-(1*H*-indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol (pindolol) 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. Thermodynamic data determination

There are two main strategies for the evaluation of the ΔG°, ΔH° and ΔS° terms. The first consists of the determination of equilibrium constants ($\Delta G^\circ = -RT \ln K_A$) in association with direct microcalorimetric measurements which give the corresponding ΔH° values. This method is not practicable in receptor binding studies because of the very low receptor concentration in most tissues (1–100 fmol/mg of tissue for a typical neurotransmitter; Bylund and Yamamura, 1990). The only method of practical use consists of measurements of K_A carried out at different temperatures, followed by van't Hoff analysis. Two cases are to 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 $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$ with $T = 298.15$ K and $R = 8.314$ JK^{-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 for the analysis (Osborne et al., 1976).

2.3. Membrane preparation

Male Wistar rats (150–200 g) were decapitated and the cortex was dissected on ice. The tissue was disrupted in a Polytron homogenizer (setting 5) in 20 volumes of 50 mM Tris-HCl, pH 7.7. The homogenate was centrifuged at $48\,000 \times g$ for 10 min and the pellet was resuspended in the same buffer. After 10 min at 37°C the membranes were centrifuged and pellets were stored at −70°C. Prior to freezing, an aliquot of homogenate was removed for protein assays (Lowry et al., 1951).

2.4. Receptor binding assays

Binding assays were performed at 0, 10, 20, 25, 30 and 35°C essentially according to Peroutka (1986): displacement experiments from 5-HT_{1A} receptors were performed in 1 ml of 50 mM Tris-HCl, pH 7.7 containing 1 nM [^{3}H]8-OH-DPAT and membranes from 8 mg (wet weight) of tissue. Non specific binding was defined as the binding in presence of 1 μM serotonin; this was always ≤ 20% of the total binding. Saturation experiments were carried out using eight different concentrations of [^{3}H]8-OH-DPAT ranging from 0.05 to 20 nM. The incubation time ranged from 60 min at 0°C to 20 min at 35°C according to previous time course experiments. All buffer solutions were adjusted to mantain a constant pH of 7.7 at any desired temperature.

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]8-OH-DPAT) derived from saturation experiments to rat cortex 5-HT_{1A} receptors; (ii) inhibitory constants, K_i (nM), for compounds **2–15** obtained by displacement of 1 nM [^{3}H]8-OH-DPAT from the same receptors

Ligand	T(K):	273	283	293	298	303	308
	t(°C):	0	10	20	25	30	35
(a) Agonists							
1. [^{3}H]8-OH-DPAT	K_D =	6.7 (±0.3)	2.7 (±0.1)	1.6 (±0.1)	0.80 (±0.03)	0.55 (±0.02)	0.74 (±0.04)
	B_{MAX} =	36.4 (±2.2)	37.8 (±2.6)	39.3 (±2.6)	36.1 (±1.4)	40.8 (±1.9)	38.5 (±2.7)
2. Serotonin		2.6 (±0.2)	3.1 (±0.2)	3.4 (±0.2)	4.4 (±0.3)	4.7 (±0.2)	5.1 (±0.4)
3. Mexamine		1.62 (±0.09)	1.94 (±0.12)	11.0 (±0.6)	13.3 (±0.7)	25.2 (±1)	29 (±2)
4. DP-5-CT		0.27 (±0.01)	0.12 (±0.01)	0.092 (±0.006)	0.074 (±0.005)	0.031 (±0.002)	0.14 (±0.01)
5. TFMPP		3 200 (±148)	930 (±59)	720 (±30)	498 (±25)	364 (±14)	459 (±26)
6. 2-MPP		608 (±24)	194 (±10)	135 (±8)	130 (±5)	59 (±3)	128 (±5)
7. *m*-CPP		217 (±9)	201 (±9)	123 (±5)	68 (±3)	68 (±4)	110 (±6)
8. CGS-12066B		695 (±39)	234 (±12)	184 (±9)	178 (±8)	68 (±3)	275 (±16)
(b) Antagonists							
9. NAN-190		13.3 (±0.9)	6.3 (±0.4)	3.1 (±0.2)	2.7 (±0.1)	1.52 (±0.06)	4.6 (±0.2)
10. Mianserin		5 230 (±240)	3 810 (±210)	3 410 (±195)	2 900 (±126)	2 330 (±112)	4 300 (±300)
11. Cyproheptadine		630 (±32)	350 (±21)	245 (±18)	244 (±10)	136 (±7)	321 (±13)
12. Ritanserin		134 000 (±5 200)	73 000 (±4 100)	11 200 (±340)	8 800 (±310)	4 700 (±200)	14 100 (±830)
13. *S*(−)-Propranolol		421 (±18)	159 (±5)	83 (±3)	77 (±4)	44 (±2)	111 (±5)
14. *S*(−)-Pindolol		150 (±8)	53 (±3)	37 (±2)	25 (±1)	18 (±1)	43 (±3)
15. Alprenolol		300 (±20)	215 (±13)	161 (±8)	156 (±7)	103 (±5)	205 (±15)

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

To determine IC_{50} values (where IC_{50} is the inhibitor concentration displacing 50% of the labelled ligand) solutions of the other ligands 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 rapid filtration through Whatman GF/B filters which were washed three times with ice cold buffer. Filter bound radioactivity was measured by scintillation spectrometry after the addition of 4 ml of Aquassure. All binding data were analysed using the non-linear regression curve fitting computer program LIGAND (Munson and Rodbard, 1990).

2.5. Calculations

For a generic binding equilibrium $L + R = LR$ (L = Ligand, R = Receptor), 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]. Inhibitory binding constants, K_i, were derived from the IC_{50} values according to the Cheng & Prusoff equation (Cheng and Prusoff, 1973) $K_i = IC_{50}/(1 + [C^*]/K_D^*)$ where $[C^*]$ is the concentration of the radioligand and K_D^* its dissociation constant. Their affinity constants, K_A, were calculated as $1/K_i$.

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. In the present case differences of standard specific heats of the equilibrium (ΔC_p°) are nearly zero and then the van't Hoff equation, $\ln K_A = \Delta S^\circ/R - \Delta H^\circ/RT$, is linear as a function of $(1/T)$ and provides standard enthalpy, ΔH°, and the standard entropy, ΔS°, values as slope and intercept, respectively. ΔG°, ΔH° and ΔS° values are given at $T = 298.15$ K.

3. Results

Table 1 reports the dissociation binding constants (K_D) and B_{MAX} values derived from the saturation experiments of [^{3}H]8-OH-DPAT performed at the six chosen temperatures; the K_D values for the other selected eleven ligands, measured as inhibitory binding constants (K_i) for the displacement of [^{3}H]8-OH-DPAT in the same range of temperature are also shown. While K_D and K_i values are largely temperature-dependent (see below), B_{MAX} values appear to be largely independent suggesting a same receptorial population at all temperatures.

Fig. 3 illustrates the representative Scatchard plots obtained at three different temperatures (0, 10, 25°C) for the saturation equilibrium of [^{3}H]8-OH-DPAT. The plots are essentially linear at all the temperatures investigated, at least in the concentration range investigated, and the computer analysis of data (Munson and Rodbard, 1990) failed to show a significantly better fit to a two-site than to a one-site binding model. Cold saturation experiments (data not shown) using [^{3}H]8-OH-DPAT 1 nM and 14–16 concentrations of unlabelled 8-OH-DPAT (0.05–100 nM) were similarly better fitted by a one-site binding model. Similar conclusions can be drawn from the analysis of the displacement curves concerning all other compounds of Table 1.

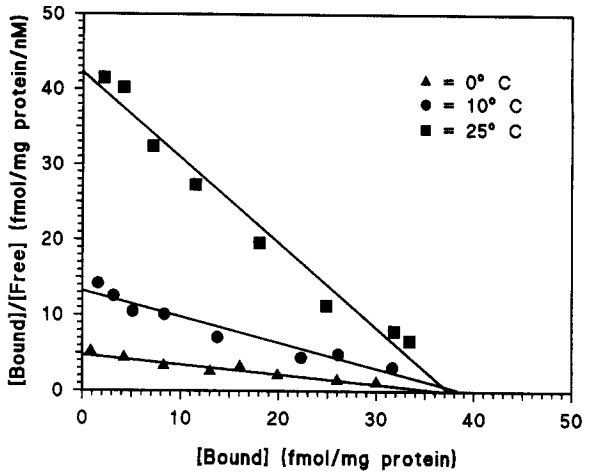

Fig. 3. Representative Scatchard plots for [^{3}H]8-OH-DPAT binding to rat cortex membranes at 0, 10 and 25°C. The linearity of the plots is indicative of the presence of a single class of high affinity binding sites at all temperatures investigated. Correlation coefficients are systematically ≥ 0.97.

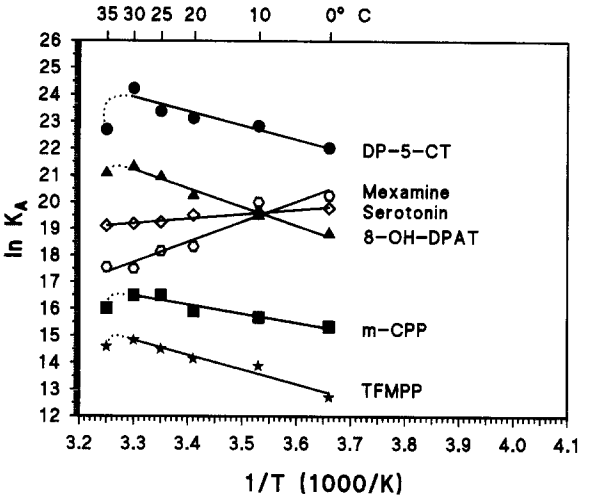

Fig. 4. Van't Hoff plots illustrating the effect of temperature on the equilibrium binding association constants, K_A, for six of the eight 5-HT_{1A} receptor agonists investigated. Linear interpolation over the points connected by the continuous line ($0 \le t \le 30$°C or $0 \le t \le 35$°C) gives correlation coefficients, r, in the range of 0.93–0.99. Curves for 2-MPP and CGS 120661, giving similar r values, have been omitted for the sake of clarity.

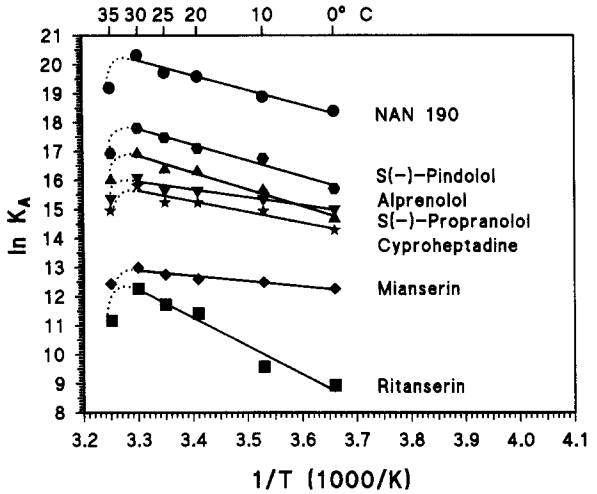

Fig. 5. Van't Hoff plots showing the effect of temperature on the equilibrium binding association constants, K_A, for the seven 5-HT_{1A} receptor antagonists investigated. Linear interpolation over the points connected by the continuous line ($0 \leq t \leq 30$°C) gives correlation coefficients, r, in the range of 0.96–0.99.

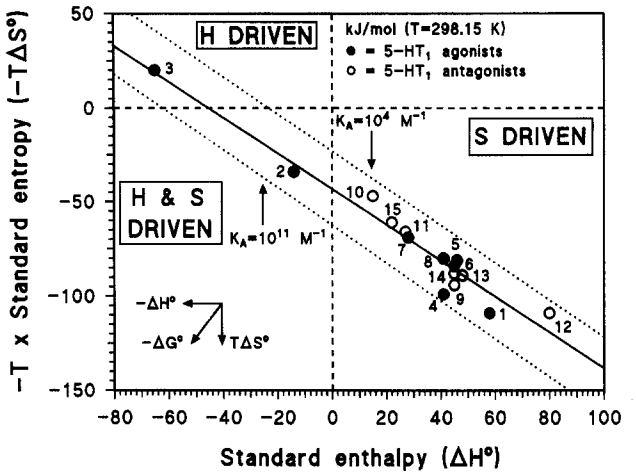

Fig. 6. $-T\Delta S°$ versus $\Delta H°$ (kJ mol^{-1}; $T = 298.15$ K) scatter plot for 5-HT_{1A} receptor agonists (full circles) and antagonists (open circles). All points lie on the same regression line (continuous line) of equation $T\Delta S° = 43(\pm 3) + 0.96(\pm 0.06)\Delta H°$ ($n = 15$, $r = -0.975$). 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). **1** = [^{3}H]8-OH-DPAT; **2** = serotonin; **3** = mexamine; **4** = DP-5-CT; **5** = TFMPP; **6** = 2-MPP; **7** = *m*-CPP; **8** = CGS-12066B; **9** = NAN-190; **10** = mianserin; **11** = cyproheptadine; **12** = ritanserin; **13** = *S*(−)-propranolol; **14** = S(−)-pindolol; **15** = alprenolol.

The temperature dependence of the affinity constants K_A is exemplified by the van't Hoff plots, ln K_A versus $1/T$ of Fig. 4 and Fig. 5, which report, respectively, data for six representative agonists (DP-5-CT, mexamine, serotonin, 8-OH-DPAT, *m*-CPP and TFMPP) and the seven antagonists (NAN 190, cyproheptadine, mianserin, ritanserin, *S*-propranolol, *S*-pindolol and alprenol) analysed. Van't Hoff plots of agonists serotonin and mexamine display positive slope (the affinity is enhanced by a decrease in temperature) and are linear between 0 and 35°C, while those of all other compounds under examination present negative slope (the affinity is enhanced by an increase in temperature) and are linear only between 0 and 30°C. The affinity constants displaying a sudden drop at 35°C.

Table 2
Thermodynamic parameters for the binding equilibrium of agonists and antagonists to rat cortex 5-HT_{1A} receptors. $\Delta G°$, $\Delta H°$ and $\Delta S°$ values are given at 298.15 K

Ligand	$\Delta G°$ (KJ mol^{-1})	$\Delta H°$ (KJ mol^{-1})	$\Delta S°$ (J K^{-1} mol^{-1})
(a) Agonists			
1. [^{3}H]8-OH-DPAT	−51.3 (±0.1)	58 (±5)	366 (±13)
2. Serotonin	−47.6 (±0.1)	−14 (±2)	113 (± 8)
3. Mexamine	−44.6 (±0.2)	−65 (±8)	−67 (±28)
4. DP-5-CT	−58.1 (±0.3)	41 (±6)	332 (±22)
5. TFMPP	−35.8 (±0.2)	46 (±4)	273 (±14)
6. 2-MPP	−39.6 (±0.1)	45 (±7)	283 (±24)
7. *m*-CPP	−40.0 (±0.1)	28 (±4)	230 (±14)
8. CGS-12066B	−39.1 (±0.2)	41 (±9)	269 (±31)
(b) Antagonists			
9. NAN-190	−49.0 (±0.1)	45 (±4)	316 (±14)
10. Mianserin	−31.5 (±0.2)	15 (±3)	156 (± 9)
11. Cyproheptadine	−38.0 (±0.2)	27 (±6)	221 (±21)
12. Ritanserin	−28.8 (±0.1)	80 (±8)	366 (±29)
13. *S*(−)-Propranolol	−40.8 (±0.2)	48 (±4)	298 (±15)
14. *S*(−)-Pindolol	−43.1 (±0.2)	45 (±4)	296 (±15)
15. Alprenolol	−38.9 (±0.2)	22 (±3)	206 (±12)

Estimated standard deviations are in parentheses.

Final thermodynamic parameters are reported in Table 2. Equilibrium standard enthalpy, $\Delta H°$, and entropy, $\Delta S°$, values show that (i) the binding of mexamine is exothermic and totally enthalpy-driven ($\Delta H° = -65$ kJ mol^{-1}, $\Delta S° = -67$ J mol^{-1} K^{-1}); (ii) the binding of the endogenous ligand serotonin is weakly exothermic and both enthalpy and entropy driven ($\Delta H° = -14$ kJ mol^{-1}, $\Delta S° = 113$ J mol^{-1} K^{-1}); (iii) the binding of all other compounds under examination is endothermic, and consequently totally entropy-driven ($15 \leq \Delta H° \leq 80$ kJ mol^{-1}, $156 \leq \Delta S° \leq 366$ J mol^{-1} K^{-1}).

Standard deviations have been estimated to be in the range 2–9 kJ mol^{-1} for $\Delta H°$ and 8–31 J mol^{-1} K^{-1} for $\Delta S°$.

The results of the thermodynamic measurements (Table 2) are summarized in the form of a $-T\Delta S°$ versus $\Delta H°$ plot (Fig. 6) already used for illustrating thermodynamic data for A_1 and A_{2a} adenosine receptors (Borea et al., 1995). All points of the plot are arranged on the same regression line following the equation

$$T\Delta S°(\text{kJ mol}^{-1}, 298.15\,\text{K}) = 43(\pm 3) + 0.96(\pm 0.06)\,\Delta H°(\text{kJ mol}^{-1})$$

$$(n = 15, r = -0.975, P < 0.01)$$

which is similar to that observed for the binding of a variety of drugs to other receptors (Gilli et al., 1994).

4. Discussion

All compounds studied display essentially linear van't Hoff plots indicating that the ΔC_p° (standard specific heat difference of the equilibrium) values of the drug-receptor binding equilibrium is nearly zero or, in other words, that ΔH° values are not significantly affected by temperature in the range investigated (0–35°C for serotonin and mexamine; 0–30°C for all the other drugs). Such linearity appears to be a typical property of the drug-membrane receptor binding (Gilli et al., 1994), unlike most binding processes between molecules and biomacromolecules occurring in solution (Sturtevant, 1977; Edsall and Gutfreund, 1983). The affinity constants for some compounds display a sudden decrease in K_A at 35°C; this phenomenon has been observed in the case of other receptors (Borea et al., 1988, 1992, 1995; Eliard and Rousseau, 1984; Dalpiaz et al., 1995) and seems to be preferably associated with endothermic drug-receptor interactions.

The scatter plot reported in Fig. 6 indicates that the drug-receptor interactions are governed by an enthalpy-entropy compensation phenomenon, which is typical of the binding processes involving biomacromolecules and has been discussed in some details by different authors (Tomlinson, 1983; Testa et al., 1987). In particular it has been recently proposed that the thermodynamic compensation phenomenon mirrors the reorganisation of water molecules during the binding (Grunwald and Steel, 1995). According to the scatter plot in Fig. 6 the binding of the two tryptamine derivatives serotonin and mexamine (Fig. 1) appears to be enthalpy driven, and at variance with the binding of all the other drugs which is totally entropy-driven. In this case agonists (full circles) and antagonists (open circles) do not appear to be thermodynamically discriminated, as they do not cluster in separate regions of the $-T\Delta S^\circ$ versus ΔH° plot, as observed in other receptor systems, i.e. β-adrenoceptor (Weiland et al., 1979), adenosine A_1 and A_2 (Borea et al., 1992; Borea et al., 1995), $GABA_A$ (Maksay, 1994) and 5-HT_3 (Borea et al., 1996). 5-HT_{1A} receptors belong therefore to the receptor family including benzodiazepines (Möhler and Richards, 1981) and D_2-dopamine (Kilpatrick et al., 1986) where agonists and antagonists cannot be distinguished on a thermodynamic bases. The thermodynamic properties of 5-HT_{1A} ligands are better interpreted in terms of their chemical characteristics.

All drugs investigated conform to the general model obtained according to SAR and conformational analysis studies, carried out by Hibert et al. (1988, Hibert et al., 1989), who have suggested that drugs must have two minimal requirements for optimal 5-HT_{1A} recognition: one aromatic nucleus and an *N*-aminic basic centre, protonated at physiological pH, at a distance of some 5 Å from it. This same model has been very recently confirmed by a detailed analysis of all available crystal structures of 5-HT_{1A} drugs covering all chemical classes displayed in Fig. 1 and Fig. 2 (with the exception of ritanserin and β-blockers; Dalpiaz et al., 1996). In spite of these 'pharmacoforic' similarities, only serotonin and mexamine have a definitely enthalpy-driven binding. Accordingly they can be distinguished from the other drugs studied by three main features which, together, may explain such exothermic binding: (i) the presence of the > NH group of the indole ring, (ii) an unsubstituted -NH_2 group as basic centre, and (iii) a hydroxyl or methoxyl substituent in indole position 5 (Fig. 1).

It seems reasonable to assume that the > NH indole group contributes to the binding of tryptamine derivatives enthalpically via a hydrogen bond accepted by the 5-HT_{1A} receptor. The same type of reasoning has been applied to the analysis of 5-HT_{2A} brain receptor binding, where it has been suggested (Dalpiaz et al., 1995) that the absence of an amino acidic residue, able to accept such a hydrogen bond in rats (Johnson et al., 1994), is the reason for the weak and strongly entropy-driven binding of serotonin.

Another drug functional group which could be involved in a strongly enthalpic interaction with the receptor could be the basic 3-ethyl-aminic function. Comparison of enthalpies for serotonin and mexamine binding with those of the other compounds (Fig. 6) would suggest a better interaction of a negatively charged function of the receptor with a -NR_2H^+ group probably because the *N*-alkyl functions hinder, at least in part, the optimal contact. This interpretation of the role played by the indolic > NH and ethylaminic NR_2H^+ fits the model proposed by Kuipers et al. (1994) for 5-HT_{1A} agonistic binding. In particular they suggest a further hydrogen bond obtained by the -OH of a threonine residue to the 5-hydroxy or methoxy functions. In this respect, thermodynamic data for serotonin and mexamine indicate that the substitution at position 5 of the hydroxyl by a methoxyl group greatly improves the enthalpic contributions to the binding (ΔH° serotonin = ± 14 kJ mol^{-1}; ΔH° mexamine = ± 65 kJ mol^{-1}). This is an indication that the 5-OH does not donate a further hydrogen bond to the receptor but just loses the OH-solvent water interaction energy (Gilli and Borea, 1991) which is not lost by the methylated substituents.

On the other hand, the binding of DP-5CT and 8-OH-DPAT (Fig. 1) is totally entropy-driven, indicating that the optimal enthalpic interaction of serotonin and mexamine is severely reduced by *N*-propyl bisubstitution. Such a substitution by the hydrophobic groups appears to produce different positive effects: besides determining a strong 5-HT_{1A} selectivity (Nelson, 1991; Zifa and Fillion, 1992) they potentiate the entropic contribution to drug-receptor binding in such a way that DP-5-CT and 8-OH-DPAT are

among the compounds endowed with the highest binding affinities (Table 1).

Also the five 1-phenyl-piperazine derivatives *m*-CPP, TFMPP, 2-MPP, NAN-190, and CGS-12066A (Fig. 1) display a totally entropy-driven binding. The best ligand is NAN-190, probably because of the large phtalimido-butyl substituent which, besides imparting antagonistic properties and high affinity for the 5-HT_{1A} receptor (Mokrosz et al., 1992; Van Steen et al., 1993, 1994), it can interact with a hydrophobic pocket of the receptor with the consequent disorganisation of solvent molecules and entropy increase. Accordingly, the three R_3 unsubstituted derivatives (*m*-CPP, TFMPP, and 2-MPP) and the methyl-substituted derivative (CGS 12066B) have binding entropies, ΔS°, with values on average of some 52 J K^{-1} mol^{-1} lower.

A dependence of ΔS° on the molecular volume is also observed in the case of the three weak antagonists mianserin, cyproheptadine and ritanserin. The last named carries very large piperidine *N*-substituents and registers the greatest ΔS° observed for all the series of compounds investigated (366 J K^{-1} mol^{-1}), confirming the absence, in this case, of specific interactions with the binding site.

The three antagonists with concomitant β-blocker activity (alprenolol, *S*(−)-propranolol and *S*(−)-pindolol) are characterized by the presence of an aromatic nucleus and an *N*-aminic centre bonded at the end of a flexible aliphatic chain which probably permits the basic centre to assume the optimal distance from the aromatic nucleus for a correct interaction at the 5-HT_{1A} receptor. The basic centre is substituted by an isopropyl group which characterizes the binding as entropy-driven and not very potent. It can be of some interest to remark that also the β-blocker activity of these compounds is characterized by a similar, from a quantitative point of view, thermodynamic profile (Weiland et al., 1979).

To conclude, the 5-HT_{1A} receptor belongs to a class of thermodynamically undiscriminated receptorial systems. Antagonist binding appears to be under entropic control. The binding of agonists is most frequently entropy-driven with the exception of the two compounds, serotonin and mexamine, where the basic centre is represented by an unsubstituted ethylamino group. In these cases the binding is performed by weak interactions and, in particular, two hydrogen bonding and an optimal interaction between charged groups have been proposed. In general it is observed that the best binders (the antagonist NAN-190 and the agonists DP-5-CT and 8-OH-DPAT) are, at the same time, the most entropy-driven and displaying the highest 5-HT_{1A} selectivity. This situation indicates that the molecular mechanism responsible for the binding of the endogenous ligand loses its fundamental importance and is substituted by hydrophobic interactions, mediated by the solvent reorganisation phenomenon, which confers high receptor affinity.

References

Boess, F.G. and I.L. Martin, 1994, Molecular biology of 5-HT receptors, Neuropharmacology 33, 275.

Borea, P.A., G.M. Bertelli and G. Gilli, 1988, Temperature dependence of the binding of μ, δ and κ agonists to opiate receptors in guinea-pig brain, Eur. J. Pharmacol. 146, 247.

Borea, P.A., K. Varani, L. Guerra, P. Gilli and G. Gilli, 1992, Binding thermodinamics of A_1 adenosine receptor ligands, Mol. Neuropharmacol. 2, 273.

Borea, P.A., A. Dalpiaz, K. Varani, L. Guerra and G. Gilli, 1995, Binding thermodinamics of A_{2a} adenosine receptor ligands, Biochem. Pharmacol. 49, 461.

Borea, P.A., A. Dalpiaz, S. Gessi and G. Gilli, 1996, Thermodynamics of 5-HT_3 receptor binding discriminates agonistic from antagonistic behaviour, Eur. J. Pharmacol. 298, 329

Bradley, P.B., G. Engel, W. Feniuk, J.R. Fozard, P.A.A. Humphrey, D.N. Middlemiss, E.J. Mylecharane, B.P. Richardson and P.R. Saxena, 1986, Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine, Neuropharmacology 25, 563.

Bylund, D.B. and H.I. Yamamura, 1990, Methods in Neurotransmitter Receptor Analysis (Raven Press, New York) p. 1.

Cheng, Y.C. and W.H. Prusoff, 1973, Relationships between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzimatic reaction, Biochem. Pharmacol. 22, 3099.

Dalpiaz, A., S. Gessi, P.A. Borea and G. Gilli, 1995, Binding thermodynamics of serotonin to rat-brain 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors, Life Sci. 57, PL 141.

Dalpiaz, A., V. Ferretti, P. Gilli and V. Bertolasi, 1996, Stereochemistry of serotonin receptor ligands from crystallographic data. Crystal structures of NAN-190. HBr, 1-Phenylbiguanide, MDL 72222 and Mianserin. HCl and selectivity criteria towards 5-HT_1, 5-HT_2 and 5-HT_3 receptor subtypes, Acta Cryst. B (in press).

Edsall, J.T. and H. Gutfreund, 1983, Biothermodynamics (J. Wiley and Sons, New York).

Eliard, H.E. and G.G. Rousseau, 1984, Thermodynamics of steroid binding to the human glucocorticoid receptor, Biochem. J. 218, 395.

Gilli, G. and P.A. Borea, 1991, Sructural chemistry and drug design, in: The Application of Charge Density to Chemistry and Drug Design (Plenum Press, New York) p. 241.

Gilli, P., V. Ferretti, G. Gilli and P.A. Borea, 1994, Enthalpy-entropy compensation in drug-receptor binding, J. Phys. Chem. 98, 1515.

Grunwald, E. and C. Steel, 1995, Solvent reorganization and thermodynamic enthalpy-entropy compensation, J. Am. Chem. Soc. 117, 5687.

Hibert, M.F., I. McDermott, D.N. Middlemiss, A.K. Mir and J.R. Fozard, 1988, Radioligand binding study of a series of 5-HT_{1A} receptor agonists and definition of a steric model of this site, Eur. J. Med. Chem. 24, 31.

Hibert, M.F., M.W. Gittos, D.N. Middlemiss, A.K. Mir and J.R. Fozard, 1989, Graphics computer-aided receptor mapping as a predictive tool for drug design: development of potent, selective, and stereospecific ligands for the 5-HT_{1A} receptor, J. Med. Chem. 31, 1087.

Hoyer, D., D.E. Clarke, J.R. Fozard, P.R. Hartig, G.P. Martin, E.J. Mylecharane, P.R. Saxena and P.A.A. Humphrey, 1994, International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), Pharmacol. Rev. 46, 157.

Johnson, M.P., R.J. Loncharich, M. Baez and D.L. Nelson, 1994, Species variation in transmemrane region V of the 5-hydroxytriptamine type 2A receptor alter the structure-activity relationships of certain ergolines and tryptamines, Mol. Pharmacol. 45, 277.

Kilpatrick, G.J., N. El Tayar, H. Van de Waterbeemd, P. Jenner, B. Testa and C.D. Marsden, 1986, The thermodynamics of agonist and antagonist binding to dopamine D-2 receptors, Mol. Pharmacol. 30, 226.

Kuipers, W., I. Van Wijngaarden and A.P. IJzerman, 1994, A Model of the Serotonin 5-HT_{1A} receptor: agonist and antagonist binding sites, Drug Design Disc. 11, 231.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the Folin Phenol reagent, J. Biol. Chem. 123, 265.

Maksay, G., 1994, Thermodynamics of γ-aminobutyric acid type A receptor binding differentiate agonists from antagonists, Mol. Pharmacol. 46, 386.

Möhler, H. and J.R. Richards, 1981, Agonist and antagonist benzodiazepine receptor interaction in vitro, Nature (London) 294, 763.

Mokrosz, J.L., M. Pietrasiewicz, B. Duszynska, M.T. Cegla, 1992, Structure-activity relationship studies of central nervous system agents. Effects of the hydrocarbon chain on the affinity 4-substituted 1(3-chlorophenyl)piperazines for 5-HT_{1A} receptor site, J. Med. Chem. 35, 2369.

Munson, P.G. and D. Rodbard D., 1990, Ligand: A versatile computerized approach for the characterization of ligand binding systems, Anal. Biochem. 107, 220.

Nelson, D.L., 1991, Structure-activity relationships at 5-HT_{1A} receptors: binding profiles and intrinsic activity, Pharmacol. Biochem. Behav. 40, 1041.

Osborne, J.P., G. Palumbo, H.B. Brewer and H. Edelhoch, 1976, The thermodynamics of the self-association of the reduced and carboxymethylated form of Apo A-II from the human high density lipoprotein complex, Biochemistry 15, 317.

Peroutka, S.J., 1986, Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} binding sites in rat frontal cortex, J. Neurochem. 47, 529.

Sturtevant, J.M., 1977, Heat capacity and entropy changes in processes involving proteins, Proc. Natl. Acad. Sci. USA 74, 2236.

Testa, B., P. Jenner, G.J. Kilpatrick, N. El Tayar, H. Van de Waterbeemd and C.D. Marsden, 1987, Do thermodynamic studies provide information on both the binding to and the activation of dopaminergic and other receptors?, Biochem. Pharmacol. 36, 4041.

Tomlinson, E., 1983, Enthalpy-entropy compensation analysis of pharmaceutical, biochemical and biological systems, Int. J. Pharm. 13, 115.

Van Steen, B.J., I. Wijngaarden, M.T.M. Tulp and W. Soudijn, 1993, Structure-affinity relationship studies on 5-HT_{1A} receptor ligands. Heterobicyclic phenylpiperazines with N4-alkyl substituents, J. Med. Chem. 36, 2751.

Van Steen, B.J., I. Wijngaarden, M.T.M. Tulp and W. Soudijn, 1994, Structre-affinity relationship studies on 5-HT_{1A} receptor ligands. Heterobicyclic phenylpiperazines with N4-aralkyl substituents, J. Med. Chem. 37, 2761.

Weiland, G.A., K.P. Minneman and P.B. Molinoff P.B., 1979, Fundamental differences between the molecolar interactions of agonists and antagonists with the β-adrenergic receptors. Nature 281, 114.

Zifa, E. and G. Fillion, 1992, 5-Hydroxytryptamine receptors, Pharmacol. Rev. 44, 401.