ELSEVIER

Biochemical Pharmacology 63 (2002) 157–161

Biochemical Pharmacology

Binding thermodynamics at the human A_3 adenosine receptor

Stefania Merighi[a], Katia Varani[a], Stefania Gessi[a], Karl-Norbert Klotz[b], Edward Leung[c], Pier Giovanni Baraldi[d], Pier Andrea Borea[a,*]

[a]*Department of Clinical and Experimental Medicine, Pharmacology Unit, Centro Nazionale di Eccellenza per lo Sviluppo di Metodologie Innovative per lo Studio ed il Trattamento delle Patologie Infiammatorie, University of Ferrara, Via Fossato di Mortara 17-19, 44100 Ferrara, Italy*

[b]*Institut für Pharmakologie und Toxikologie, Universität Würzburg, Würzburg, Germany*

[c]*Medco Research, Research Triangle Park, Triangle Park, USA*

[d]*Department of Pharmaceutical Sciences; University of Ferrara, Ferrara, Italy*

Received 23 May 2001; accepted 25 September 2001

Abstract

The thermodynamic parameters ΔG°, ΔH° and ΔS° of the binding equilibrium of six adenosine receptor agonists and five antagonists at adenosine A_3 receptors were determined by means of affinity measurements at six different temperatures (4, 10, 15, 20, 25 and 30°) and van't Hoff plots were constructed. Affinity constants were measured on Chinese hamster ovary (CHO) cells transfected with the human A_3 receptors by inhibition assays of the binding of the selective A_3 antagonist [^{3}H]MRE 3008F20. van't Hoff plots were linear for agonists and antagonists in the temperature range 4–30°. Their thermodynamic parameters fall in the ranges $21 \leq \Delta H^\circ \leq 67$ kJ mol^{-1} and $208 \leq \Delta S^\circ \leq 410$ J (K mol)$^{-1}$ for agonists and $-52 \leq \Delta H^\circ \leq -9$ kJ mol^{-1} and $16 \leq \Delta S^\circ \leq 81$ J (K/mol)$^{-1}$ for antagonists, showing that agonist binding is always totally entropy-driven while antagonist binding is enthalpy- and entropy-driven. The results are discussed with the aim of obtaining new details on the nature of the forces driving the A_3 binding at a molecular level. © 2002 Published by Elsevier Science Inc.

Keywords: Binding thermodynamics; Adenosine A_3 receptor; Enthalpy–entropy compensation; Agonist–antagonist discrimination

1. Introduction

The thermodynamic analysis of the binding equilibrium of a drug to its receptor allows us to evaluate the two components, standard enthalpy (ΔH°) and standard entropy (ΔS°), of the standard free energy (ΔG°) of the binding equilibrium [1]. It is often assumed that ΔH° and ΔS° terms represent the two classes of factors responsible for the drug–receptor recognition phenomenon: non-bonded interactions, as hydrogen bonding and multipolar or dispersive interactions (which are mainly to be related to the enthalpic term), and solvent reorganization (which is most properly associated with the entropic one) [2]. Until now, such studies have been carried out on several receptor systems and critical appraisals of the method also exist in the literature [3,4].

Adenosine is an endogenous modulator acting as an autacoid via interaction with four types of G protein-coupled receptors: A_1, A_{2A}, A_{2B} and A_3 [5]. A_3 receptors are widely distributed in human body suggesting their involvement in regulating many physiological functions [6,7]. Recently, we have performed a pharmacological and biochemical characterization of [^{3}H]MRE 3008F20 binding to the human A_3 receptors transfected in CHO cells [8].

* Corresponding author. Tel.: +39-532-291-214; fax: +39-532-291-205.
E-mail address: bpa@dns.unife.it (P.A. Borea).

Abbreviations: [^{3}H]MRE 3008F20, [^{3}H]5-*N*(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine; NECA, 5′-*N*-ethyl-carboxamidoadenosine; R-PIA, R(−)N^6(2-phenyl-isopropyl)adenosine; S-PIA, S(−)N^6(2-phenylisopropyl)adenosine; Cl-IB-MECA, N^6(3-iodobenzyl)-2-chloroadenosine-5′-*N*-methyluronamide; IB-MECA, N^6(3-iodobenzyl)adenosine-5′-*N*-methyluronamide; AB-MECA, 4-aminobenzyl-5′-*N*-methylcarboxamidoadenosine; CGS 15943, 5-amino-9-chloro-2-(furyl)-1,2,4-triazolo[1,5-*c*]quinazoline; DPCPX, 1,3-dipropyl-8-cyclopentyl-xanthine; SCH 58261, 7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine; MRE 3008F20, 5*N*(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; MRE 3020F20, 5*N*(3-chlorophenylcarbamoyl)amino-8-ethyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; MRE 3005F20, 5*N*(4-methoxyphenylcarbamoyl)amino-8-phenylethyl-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; PBS, phosphate buffered saline; hCHO-A_3, CHO cells transfected with human A_3 adenosine receptor.

0006-2952/02/$ – see front matter © 2002 Published by Elsevier Science Inc.
PII: S0006-2952(01)00825-5

Furthermore, we have shown that MRE 3008F20 binding to human A_3 receptors is enthalpy- and entropy-driven [8]. A_3 receptors exist in equilibrium between two different conformations, which correspond to a high and a low affinity binding state; antagonists bind equally well to both receptor states whereas agonists discriminate between them [9–12]. With the aim of contributing to an evaluation of the molecular mechanism underlying ligand–receptor interactions, this paper reports the results derived from the study of binding thermodynamics of six agonists and five antagonists to human A_3 receptors transfected in CHO cells. High and low affinity states of the receptors were analyzed from a thermodynamic point of view. The results are compared with those obtained for the related A_1 and A_{2A} receptors [13,14], providing useful information on the binding mechanisms of all adenosine receptor subtypes.

2. Materials and methods

2.1. Materials

[^{3}H]MRE 3008F20 (specific activity 67 Ci mmol^{-1}) was obtained from Amersham International. AB-MECA, NECA, R-PIA, S-PIA, Cl-IB-MECA, IB-MECA, CGS 15943, DPCPX were obtained from Research Biochemical International. SCH 58261, MRE 3020F20, MRE 3005F20, MRE 3008F20 were synthesized by P.G. Baraldi, University of Ferrara. CHO cells transfected with the human recombinant A_3 adenosine receptor (hCHO-A_3) were obtained by K.-N. Klotz, University of Wurzburg, as described earlier [15]. All other reagents were of analytical grade and obtained from commercial sources.

2.2. Cell culture

hCHO-A_3 were grown adherently and maintained in Dulbecco's Modified Eagle's Medium with nutrient mixture F12 (DMEM/F12), containing 10% fetal calf serum, penicillin (100 U mL^{-1}), streptomycin (100 μg mL^{-1}), L-glutamine (2 mM) and Geneticin (G418, 0.2 mg mL^{-1}) at 37° in 5% CO_2/95% air. Cells were split 2 or 3 times weekly at a ratio between 1:5 and 1:20.

2.3. Membrane preparation

For membrane preparation, the culture medium was removed. The cells were washed with PBS and scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris–HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with Polytron and the homogenate was spun for 10 min at 1000 *g*. The supernatant was then centrifuged for 30 min at 100,000 *g*. The membrane pellet was resuspended in 50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM EDTA and incubated with 3 IU mL^{-1} of adenosine deaminase for 30 min at 37°. Then the suspension was frozen at −80°.

2.4. Receptor binding assays

Binding assays were performed on hCHO-A_3 membranes at 4, 10, 15, 20, 25 and 30°, in a thermostatic bath assuring a temperature of ±0.1°. All buffer solutions were adjusted to maintain a constant pH of 7.4 at the desired temperature. Competition experiments of 1 nM [^{3}H]MRE 3008F20 were performed in duplicate in a final volume of 100 μL in test tubes containing 50 mM Tris–HCl buffer, 10 mM $MgCl_2$, 1 mM EDTA and 100 μL of membranes and at least 12–14 different concentrations of typical adenosine receptor agonists and antagonists. Non-specific binding was defined as binding in the presence of 1 μM MRE 3008F20 and was about 30% of total binding. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass-fiber filters using a Micro-Mate 196 cell harvester (Packard Instrument Company). The filter bound radioactivity was counted using a Microplate Scintillation Counter (Top Count, Meriden, CT) at an efficiency of 57% with Micro-Scint 20. The protein concentration was determined according to a Bio-Rad method [16] with bovine albumin as a standard reference. Incubation time ranged from 150 min at 4° to 60 min at 30° according to the results of previous time-course experiments. All binding data were analyzed using the non-linear regression curve-fitting computer program LIGAND [17].

2.5. Thermodynamic data determination

Determination of ΔG°, ΔH° and ΔS° values has been obtained by measurements of K_A (equilibrium association constant) 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 essentially zero. In this case, the van't Hoff equation $\ln K_A = -\Delta H^\circ/RT + \Delta S^\circ/R$ gives a linear plot $\ln K_A$ vs. $1/T$. The standard free energy can be calculated as $\Delta G^\circ = -RT \ln K_A$ at 298.15 K; the standard enthalpy (ΔH°) and the standard entropy (ΔS°) can be obtained from the slope ($-\Delta H^\circ/R$) and the intercept ($\Delta S^\circ/R$) of the van't Hoff plot $\ln K_A$ vs. $1/T$, respectively, with $R = 8.314$ J (K mol)$^{-1}$. The linearity of van't Hoff plots is not common in reactions involving biomacromolecules in solution but appears to be typical as far as membrane receptor binding is concerned [18]. (2) ΔC_p° is not equal to zero [19,20].

3. Results

Table 1 reports the inhibitory binding constants K_i for agonists and antagonists at human A_3 adenosine receptors stably expressed in CHO cells. These values were determined at various temperatures by displacement of the radiolabeled antagonist [^{3}H]MRE 3008F20. The analysis of the inhibition curves of agonists revealed two affinity states, which is a typical result of experiments performed in

Table 1
Affinities, expressed as K_H, K_L and K_i, values (nM), of selected adenosine receptor agonists and antagonists to human A_3 cloned receptors expressed in CHO cells[a]

Ligand T (K)		277.15	283.15	288.15	293.15	298.15	303.15
Agonists							
IB-MECA	K_H	1.5 ± 0.2	0.76 ± 0.02	0.43 ± 0.02	0.40 ± 0.06	0.19 ± 0.01	0.11 ± 0.01
	K_L	760 ± 45	504 ± 65	672 ± 49	511 ± 69	359 ± 42	250 ± 32
Cl-IB-MECA	K_H	1.6 ± 0.2	0.8 ± 0.1	0.58 ± 0.02	0.45 ± 0.02	0.43 ± 0.01	0.23 ± 0.01
	K_L	494 ± 40	372 ± 25	248 ± 22	131 ± 15	284 ± 30	110 ± 12
AB-MECA	K_H	12 ± 1	9.7 ± 0.1	8.0 ± 0.3	7.8 ± 0.4	5.0 ± 0.5	6.3 ± 0.2
	K_L	4480 ± 520	3600 ± 410	2131 ± 311	1181 ± 185	1220 ± 160	1093 ± 172
NECA	K_H	30 ± 3	27 ± 3	24 ± 2	20 ± 3	19 ± 2	11 ± 1
	K_L	3700 ± 480	2719 ± 310	2132 ± 250	1744 ± 185	1238 ± 110	1302 ± 120
R-PIA	K_H	66 ± 6	55 ± 5	42 ± 6	24 ± 3	31 ± 4	21 ± 3
	K_L	8032 ± 900	5202 ± 600	4480 ± 500	3600 ± 330	2930 ± 325	2447 ± 254
S-PIA	K_H	158 ± 18	104 ± 12	68 ± 9	56 ± 4	69 ± 5	44 ± 6
	K_L	ND	ND	ND	ND	ND	ND
Antagonists							
MRE 3008F20	K_i	0.85 ± 0.02	1.2 ± 0.2	1.8 ± 0.2	2.1 ± 0.2	2.9 ± 0.1	3.3 ± 0.2
MRE 3020F20	K_i	2.1 ± 0.2	3.1 ± 0.2	4.1 ± 0.2	6.3 ± 0.4	7.0 ± 0.5	9.9 ± 0.4
MRE 3005F20	K_i	2.7 ± 0.3	3.8 ± 0.4	5.7 ± 0.4	12 ± 1	16 ± 2	14 ± 1
CGS 15943	K_i	71 ± 4	77 ± 8	86 ± 5	95 ± 10	109 ± 13	125 ± 12
DPCPX	K_i	1407 ± 150	1501 ± 170	1538 ± 140	1720 ± 185	1882 ± 190	1955 ± 200
SCH 58261	K_i	>10000	>10000	>10000	>10000	>10000	>10000

[a] Values are the mean ± SEM of four separate experiments performed in triplicate. ND: not detectable.

membrane preparations in the absence of GTP with an antagonist as radioligand. Our experimental conditions permit us to obtain ≈30% of adenosine A_3 receptors in the high affinity state [8]. High and low affinity sites were detected at all temperatures. The only exception was S-PIA for which the low affinity site could not be assessed at a reasonable level of accuracy. Fig. 1(A and B) illustrates the van't Hoff plots, ln K_A vs. $1/T$, for agonists and antagonists, respectively. All plots appear to be linear in the full temperature range (4–30°). In particular, the slopes are systematically negative for agonists and positive for antagonists. Fig. 2 summarizes the results in the form of a $-T\,\Delta S^\circ$ vs. ΔH° scatter plot ($T = 298$ K). It becomes apparent that all points are arranged on the same diagonal band encompassed between the two dashed lines which represent the loci of points defined by the limiting K_D values of 100 μM and 100 pM. This band is the expression of the enthalpy–entropy (*E–E*) compensation phenomenon [18]. Final thermodynamic parameters of the different compounds investigated are reported in Table 2. ΔG° values range from −55.2 to −26.8 kJ mol^{-1} for agonists and from −48.8 to −32.6 kJ mol^{-1} for antagonists. Equilibrium standard enthalpy (ΔH°) and entropy (ΔS°) values show that the binding of agonists is always totally entropy-driven while is enthalpy- and entropy-driven for antagonists, ΔH° values ranging from 21 to 67 and from −52 to −9 kJ mol^{-1} and ΔS° values from 208 to 410 J (K mol)$^{-1}$ and from 16 to 81 J (K mol)$^{-1}$ for agonists and antagonists, respectively. Agonists and antagonists are therefore thermodynamically discriminated.

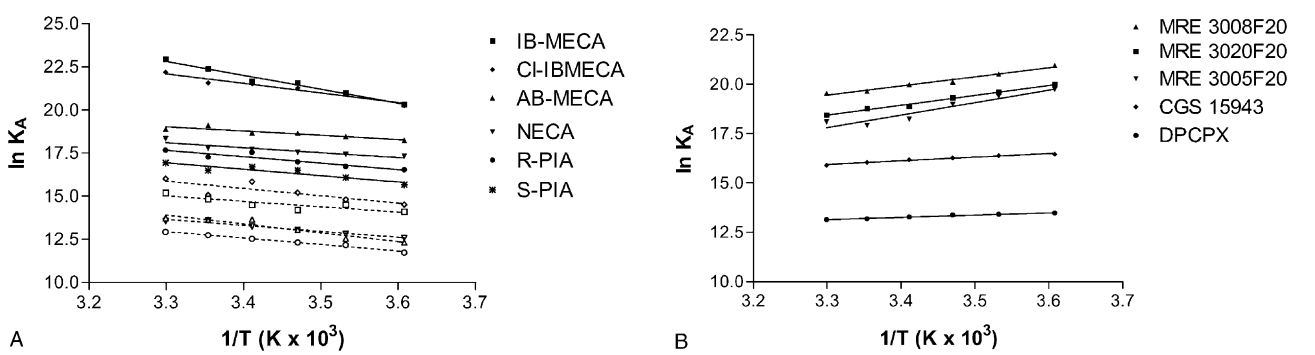

Fig. 1. van't Hoff plots showing the effect of temperature on the equilibrium binding association constants, K_A, for all adenosine A_3 receptor agonists (A) and antagonists (B) studied. All plots are essentially linear ($r \geq 0.91$) in the temperature range of 4–30°. Continuous or dashed lines are for high or low affinity binding constants, respectively.

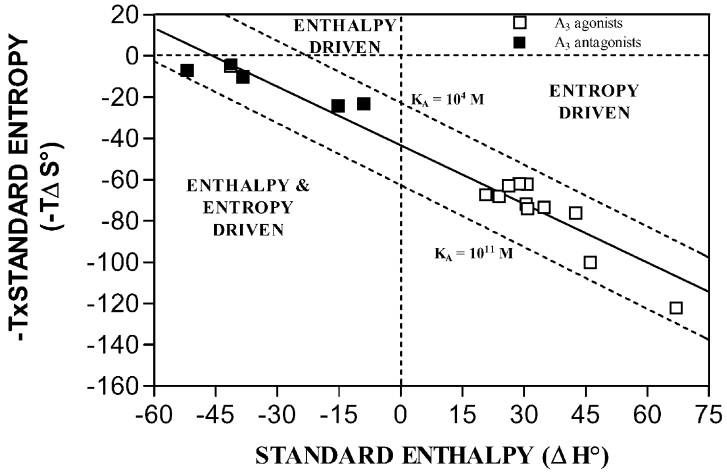

Fig. 2. Scatter plot of $-T\,\Delta S^\circ$ vs. ΔH° values for the adenosine A_3 receptor ligands studied. Full and open symbols indicate antagonists and agonists, respectively. All points lie on a same regression line. The two dashed lines indicate the *loci* of the points representing possible combinations of ΔH° and $-T\,\Delta S^\circ$ values giving rise to the two different equilibrium constants indicated ($K_A = 10^4$ and $K_A = 10^{11}$).

Table 2
Thermodynamic parameters for displacement of [^{3}H]MRE 3008F20, by adenosine agonists and antagonists from human A_3 cloned receptors expressed in CHO cells[a]

Ligand		ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J (K mol)$^{-1}$)
Agonists				
IB-MECA	K_H	−55.2 ± 0.2	67 ± 5	410 ± 8
	K_L	−36.6 ± 0.1	26 ± 2	211 ± 7
Cl-IB-MECA	K_H	−53.8 ± 0.1	46 ± 3	335 ± 5
	K_L	−38.6 ± 0.1	35 ± 1	246 ± 7
AB-MECA	K_H	−46.6 ± 0.2	21 ± 1	225 ± 4
	K_L	−33.6 ± 0.3	42 ± 3	255 ± 6
NECA	K_H	−44.3 ± 0.1	24 ± 2	228 ± 9
	K_L	−33.2 ± 0.2	29 ± 1	208 ± 1
R-PIA	K_H	−43.1 ± 0.2	31 ± 2	248 ± 7
	K_L	−31.4 ± 0.1	31 ± 3	208 ± 8
S-PIA	K_H	−41.3 ± 0.3	30 ± 1	241 ± 5
	K_L	ND	ND	ND
Antagonists				
MRE 3008F20	K_i	−48.7 ± 0.2	−38 ± 1	34 ± 4
MRE 3020F20	K_i	−46.2 ± 0.1	−41 ± 2	16 ± 2
MRE 3005F20	K_i	−44.8 ± 0.2	−52 ± 2	24 ± 2
CGS 15943	K_i	−39.6 ± 0.1	−15 ± 1	81 ± 6
DPCPX	K_i	−32.6 ± 0.2	−9 ± 1	78 ± 8
SCH 58261	K_i	ND	ND	ND

[a] Equilibrium ΔG°, ΔH° and ΔS° values are given at $T = 298.15$ K. ND: not detectable.

4. Discussion

The most significant results of this paper are: (i) the systematic linearities of van't Hoff plots; (ii) the recurrent phenomenon of *E–E* compensation; (iii) the thermodynamic discrimination of agonists from antagonists. Regarding the first point, van't Hoff plots turn out to be linear for all compounds considered. This implies that the ΔC_p° for the binding equilibrium approximates zero in all cases, or, in other words, the value of ΔH° is not significantly affected by temperature variations in the range investigated (4–30°) [21]. This phenomenon seems to indicate that the conformational changes needed to produce the pharmacological effect are relatively small in this class of macromolecules, most probably because larger modifications would make the association of the receptor with the cell membrane unstable. This point of view is strongly supported by the finding that all membrane receptors so far studied are characterized by essentially linear van't Hoff plots [18] with the only known exception of the binding of insulin to its receptor [22]. The second point concerns the extrathermodynamic interdependence of ΔH° and $-T\,\Delta S^\circ$ for the A_3 receptor, clearly apparent from Fig. 2, where all the experimental points appear to be arranged along a same diagonal line, according to the equation $-T\,\Delta S^\circ$ (kJ mol^{-1} at 298.15 K) $= -42\ (\pm 2) - 0.95\ (\pm 0.05)\ \Delta H^\circ$ (kJ mol^{-1}), ($n = 19$, $r = -0.976$, $P < 0.0001$). This equation can be rewritten as $\Delta H^\circ = \beta \Delta S^\circ$, which is the form for a case of enthalpy–entropy compensation [18,23] with a compensation temperature of 302 K. This phenomenon seems to be a common feature in all cases of drug–receptor binding [18] and its general implications have been discussed by different authors [23,24]. Recently, 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 [23]. According to this point of view, while ΔG° values are most probably determined by the features of the ligand–receptor binding process, ΔH° and $-T\,\Delta S^\circ$ values appear strongly affected by the rearrangements occurring in the solvent [23]. Last, but not least, we observed that the binding equilibrium of A_3 receptor ligands is entropy-driven for agonists, but mostly enthalpy-driven for antagonists. Agonists and antagonists are thermodynamically discriminated for all adenosine receptors studied so far [13]. For both A_1 and A_{2A} receptors it has been shown that agonist binding

is entropy-driven while antagonist binding is mainly enthalpy-driven. In this light we suggest, for the first time, that the similarity in thermodynamic parameters probably reflects a common mechanism of ligand–receptor interaction for all adenosine receptor subtypes. For the sake of completeness, it should be noted that thermodynamic studies for the A_1 and A_{2A} receptor were conducted on rat cortex membranes while the A_3 subtype was investigated in hCHO-A_3. For this reason, this suggestion has to be considered with the due caution. However, this finding may be the key for understanding the difficulty to synthesize selective adenosine receptor ligands. Although much progress has been made in recent years to obtain potent and selective adenosine receptor ligands, at present compounds exhibiting high affinity to only one subtype are an exception [25,26].

References

[1] Hitzemann R. Thermodynamics aspects of drug–receptor interactions. Trends Pharmacol Sci 1988;9:408–11.

[2] Gilli G, Borea PA, The application of charge density to chemistry and drug-design. In: Jeffrey GA and Piniella JF, editors. New York: Plenum Press, 1991. p. 241–86.

[3] Testa B, Jenner P, Kilpatrick GJ, El Tayar N, van de Waterbeemd H, Marsden CD. Do thermodynamic studies provide information on both the binding to and the activation of dopaminergic and other receptors? Biochem Pharmacol 1987;36:4041–6.

[4] Raffa RB, Porreca F. Thermodynamic analysis of the drug–receptor interaction. Life Sci 1989;44:245–58.

[5] Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden K, Jacobson KA, Leff P, Williams M. Nomenclature and classification of purinoceptors. Physiol Rev 1994;46:143–56.

[6] Jacobson KA. Adenosine A_3 receptors: novel ligands and paradoxical effects. Trends Pharmacol Sci 1998;19:184–91.

[7] Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol Rev 1998;50:413–92.

[8] Varani K, Merighi S, Gessi S, Klotz K-N, Leung E, Baraldi PG, Cacciari B, Romagnoli R, Spalluto G, Borea PA. [^{3}H]MRE 3008F20: a novel antagonist radioligand for the pharmacological and biochemical characterization of human A_3 adenosine receptors. Mol Pharmacol 2000;57:968–75.

[9] Lohse MJ, Lenschow V, Schwabe U. Two affinity states of Ri adenosine receptors in brain membranes: analysis of guanine nucleotide and temperature effects on radioligand binding. Mol Pharmacol 1984;26:1–9.

[10] Milligan G, Bond RA, Lee M. Inverse agonism: pharmacological curiosity or potential therapeutic strategy? Trends Pharmacol Sci 1995;16:10–3.

[11] Dalpiaz A, Townsend-Nicholson A, Beukers MW, Schofield PR, IJzerman AP. Thermodynamics of full agonist, partial agonist, and antagonist binding to wild-type and mutant adenosine A_1 receptors. Biochem Pharmacol 1998;56:1437–45.

[12] Lorenzen A, Guerra L, Vogt H, Schwabe U. Interaction of full and partial agonists of the A_1 adenosine receptor with receptor/G protein complexes in rat brain membranes. Mol Pharmacol 1996;49:915–26.

[13] Borea PA, Dalpiaz A, Varani K, Gessi S, Gilli G. Binding thermodynamics at A_1 and A_{2A} adenosine receptors. Life Sci 1996;59:1373–88.

[14] Lorenzen A, Guerra L, Campi F, Lang H, Schwabe U, Borea PA. Thermodynamically distinct high and low affinity states of the A_1 adenosine receptor induced by G protein coupling and guanine nucleotide ligation states of G proteins. Br J Pharmacol 2000;130:595–604.

[15] Klotz K-N, Hessling J, Hegler J, Owman C, Kull B, Fredholm BB, Lohse MJ. Comparative pharmacology of human adenosine receptor subtypes—characterization of stably transfected receptors in CHO cells. Naunyn Schmiedebergs Arch Pharmacol 1998;357:1–9.

[16] Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye-binding. Anal Biochem 1976;72:248–54.

[17] Munson PJ, Rodbard D. LIGAND: a versatile computerized approach for the characterization of ligand binding systems. Anal Biochem 1980;107:220–39.

[18] Gilli P, Ferretti V, Gilli G, Borea PA. Enthalpy–entropy compensation in drug–receptor binding. J Phys Chem 1994;98:1515–8.

[19] Eliard PH, Rousseau GG. Thermodynamics of steroid binding to the human glucocorticoid receptor. Biochem J 1984;218:395–404.

[20] Borea PA, Varani K, Gessi S, Gilli P, Gilli G. Binding thermodynamics at the human neuronal nicotine receptor. Biochem Pharmacol 1998;55:1189–97.

[21] Dalpiaz A, Scatturin A, Pavan B, Varani K, IJzerman AP, Borea PA. Thermodynamic in vitro studies as a method to investigate the pharmacodynamic behavior of adenosine A_1 receptor ligands. Pharmacol Res 1999;16:1054–8.

[22] Waelbroeck M, Van Obberghen E, De Meyts P. Thermodynamics of the interaction of insulin with its receptor. J Biol Chem 1979;254:7736–40.

[23] Tomlinson E. Enthalpy–entropy compensation analysis of pharmaceutical, biochemical and biological systems. Int J Pharmaceutics 1983;13:115–44.

[24] Grunwald E, Steel C. Solvent reorganization and thermodynamic enthalpy–entropy compensation. J Am Chem Soc 1995;117:5687–92.

[25] Klotz K-N. Adenosine receptors and their ligands. Naunyn Schmiedebergs Arch Pharmacol 2000;362:382–91.

[26] Baraldi PG, Borea PA. New potent and selective human adenosine A_3 receptor antagonists. Trends Pharmacol Sci 2000;21:456–9.