0026-895X/94/020386-05$03.00/0

MOLECULAR PHARMACOLOGY, 46:386-390, 1994.

Thermodynamics of γ-Aminobutyric Acid Type A Receptor Binding Differentiate Agonists from Antagonists

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Received January 10, 1994; Accepted May 18, 1994

SUMMARY

Specific binding of the γ-aminobutyric acid $(GABA)_A$ antagonist [^{3}H]SR 95531 to synaptosomal membranes of rat whole brain was examined between 0° and 37°. Scatchard analysis revealed two (high and low affinity) populations of [^{3}H]SR 95531 binding sites. The K_d values increased with increasing temperature. K_i values for $GABA_A$ agonists and antagonists were determined from the displacement of [^{3}H]SR 95531 binding at a low concentration (1.8 nM) of [^{3}H]SR 95531, which binds predominantly to high affinity sites. For most compounds van't Hoff plots ($-\ln K_i$, i.e., $\ln K_a$, versus $1/T$) were linear between 0° and 37°. Curvilinear van't Hoff plots for the antagonists R 5135 and bicuculline methiodide can be attributed to their hydrophobic binding interactions. The enthalpy changes of binding ($\Delta H°$) were positive for the agonists (muscimol, isoguvacine, GABA, 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol hydrochloride, and imidazole-4-acetic acid) and negative for the antagonists (pitrazepin, bicuculline methiodide, R 5135, SR 95531, and SR 95103). Separation of the enthalpic and entropic components of the Gibbs free energy changes of binding ($\Delta G°$) revealed that binding of the antagonists is driven by both the enthalpic and entropic terms, whereas that of the agonists is driven entirely by entropy changes. A plot of the entropic term ($-T\Delta S°$) versus the enthalpic term ($\Delta H°$) showed separate patterns for $GABA_A$ agonists and antagonists, with the partial agonists [5-(4-piperidyl)isoxazol-3-ol, imidazole-4-acetic acid, and 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol hydrochloride] between them. It is proposed that the entropic term is partly determined by a transition from antagonist to agonist conformation of the $GABA_A$ binding sites.

GABA is a major inhibitory neurotransmitter in mammalian brain. Its binding to $GABA_A$ receptors opens an attached chloride channel (1). The thermodynamic parameters of this interaction can be determined from the temperature dependence of receptor binding. Weiland *et al.* (2) have reported a correlation between the efficacies and thermodynamic parameters of binding to β-adrenergic receptors. Although this correlation has been confirmed (3), its validity for other receptor systems has been questioned (4). Similar studies have been performed for various receptors, including strychnine-sensitive glycine receptors (5) and the benzodiazepine binding site of the $GABA_A$ receptor complex (6–8). The thermodynamic parameters of binding did not correlate with the efficacies of benzodiazepine agonists and inverse agonists to modulate GABAergic neurotransmission (8). No such studies have been reported for the GABA binding sites of the $GABA_A$ receptor complex, in spite of its utmost importance. This shortage of information might be attributed to technical difficulties. A heterogeneous population of $GABA_A$ receptor sites exists (1) and their radioligands rapidly dissociate from them. The pioneering study of sodium-independent binding of [^{3}H]GABA showed a flat optimum between 15 and 25° (9). Specific binding of the $GABA_A$ antagonist [^{3}H]bicuculline methiodide increased (10), whereas that of another antagonist, [^{3}H]SR 95531, decreased with increasing temperature (11). These anomalies justified a detailed study of the temperature dependence of $GABA_A$ receptor binding.

[^{3}H]Bicuculline methochloride (12) and [^{3}H]SR 95531 (13, 14) bind preferentially to the low affinity (micromolar) agonist sites of $GABA_A$ receptors. Regional distribution of [^{3}H]SR 95531 binding in rat brain showed good correspondence with [^{3}H]GABA binding, with the exception of cerebellum and hippocampus (15). SR 95531 selectively prevented photoaffinity labeling of the 58-kDa β3 subunit of $GABA_A$ receptors with [^{3}H]muscimol (16). Therefore, high affinity [^{3}H]SR 95531 binding was used to characterize the thermodynamic driving forces of $GABA_A$ receptor binding.

Experimental Procedures

Materials. [^{3}H]SR 95531 (49 Ci/mmol) was obtained from New England Nuclear. 4-PIOL·HBr, THIP, and isoguvacine·HCl were kindly donated by Prof. P. Krogsgaard-Larsen (Royal Danish School of Pharmacy, Copenhagen, Denmark), SR 95531 and SR 95103 by SANOFI (Montpellier, France), pitrazepin by Sandoz (Bern, Switzer-

This work was funded by Grant OTKA 1763 from the Hungarian Science Research Fund.

ABBREVIATIONS: GABA, γ-aminobutyric acid; 4-PIOL, 5-(4-piperidyl)isoxazol-3-ol; R 5135, 3α-hydroxy-16-imino-5β,17-aza-androstan-11-one; SR 95103, 2-(3-carboxypropyl)-3-amino-4-methyl-6-phenylpyridazinium chloride; SR 95531, 2-(3-carboxypropyl)-3-amino-6-*p*-methoxyphenylpyridazinium bromide; THIP, 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol hydrochloride.

land), and R 5135 by Dr. P. Hunt (Roussel-Uclaf, Romainville, France). Muscimol was obtained from Serva, and bicuculline methiodide and imidazole-4-acetic acid·HCl were from Sigma.

Membrane preparation. Extensively washed freeze-thawed membranes were prepared from whole brains of male Wistar rats as described (13). In brief, brains were homogenized in 0.32 M sucrose and centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged at 20,000 × *g* for 20 min. The pellet was dispersed in distilled water with an Ultra-Turrax homogenizer for 15 sec and was centrifuged at 8000 × *g* for 20 min. The supernatant and the buffy coat of the pellet were centrifuged at 40,000 × *g* for 20 min. The pellet was washed twice by similar centrifugations in distilled water and frozen. The next day the membranes were thawed, suspended in 50 mM Tris-citrate buffer, pH 7.1, centrifuged at 40,000 × *g* for 10 min, and frozen. Before the binding assay, the suspension was thawed and centrifuged in 50 mM Tris-citrate buffer.

Binding studies. For saturation analysis, membrane suspensions in 50 mM Tris-citrate buffer (0.4 mg protein/ml) were incubated with 3 nM [^{3}H]SR 95531 and nine concentrations of SR 95531 (up to 800 nM) simultaneously at five temperatures between 0° and 37° (homologous displacement). Incubations were terminated after 20 min (37°) to 50 min (0°) by filtration of two 0.45-ml aliquots on Whatman GF/B filters under vacuum and rapid rinsing with 2 × 3 ml of cold buffer. Nonspecific binding was determined in the presence of 1 mM GABA. For determination of IC_{50} values, five to seven concentrations of GABAergic agents and 1.8 nM [^{3}H]SR 95531 were incubated with the membranes. Protein concentrations were determined using the Bio-Rad reaction (17), with bovine serum albumin as standard.

Data analysis. Nonlinear regression of the binding data with the program LIGAND (18) resulted in K_d and B_{max} values for two populations of binding sites. K_i values of GABAergic displacing agents were determined according to eq. 1,

$$K_i = IC_{50}/(1 + c/K_{d1}) \quad (1)$$

where c = 1.8 nM [^{3}H]SR 95531 and K_{d1} is the dissociation constant for high affinity [^{3}H]SR 95531 binding (see Table 1). Linear van't Hoff plots ($-\ln K_i$ versus $1/T$) result in slope values of $-\Delta H^\circ/R$. Gibbs free energy changes were calculated at 37° according to eq. 2,

$$\Delta G^\circ = -RT \cdot \ln K_i = RT \cdot \ln K_a \quad (2)$$

where $K_a = K_i^{-1}$. Entropy changes of binding (ΔS°) were determined from the thermodynamic data at 37° according to eq. 3.

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (3)$$

When the van't Hoff plots were not linear, ΔG° values were expressed as a polynomial function of T according to eq. 4 (19).

$$\Delta G^\circ = RT \cdot \ln K_i = A + BT + CT^2 \quad (4)$$

The ΔH°, ΔS°, and heat capacity changes (ΔC_p°) can be calculated from the regression coefficients according to eqs. 5–7.

$$\Delta H^\circ = A - CT^2 \quad (5)$$

$$\Delta S^\circ = -B - 2CT \quad (6)$$

$$\Delta C_p^\circ = -2CT \quad (7)$$

Note that the thermodynamic parameters are standardized to 37° rather than 25°.

Results and Discussion

The specific binding of [^{3}H]SR 95531 was studied at five temperatures between 0° and 37°. The displacing potencies of SR 95531 decreased with increasing temperature (Fig. 1). The homologous displacement data of Fig. 1 fitted by LIGAND (18) revealed two populations of binding sites (Table 1), as reported (11). Increasing the temperature from 0° to 37° increased the K_{d1} values by 2–3-fold. Because the B_{max} values of SR 95531 binding did not decrease with increasing temperature (Table 1), the filtration method could be reliably used up to 37°. All subsequent displacement studies were performed with a low concentration (1.8 nM) of [^{3}H]SR 95531, where the contribution of high affinity binding predominates.

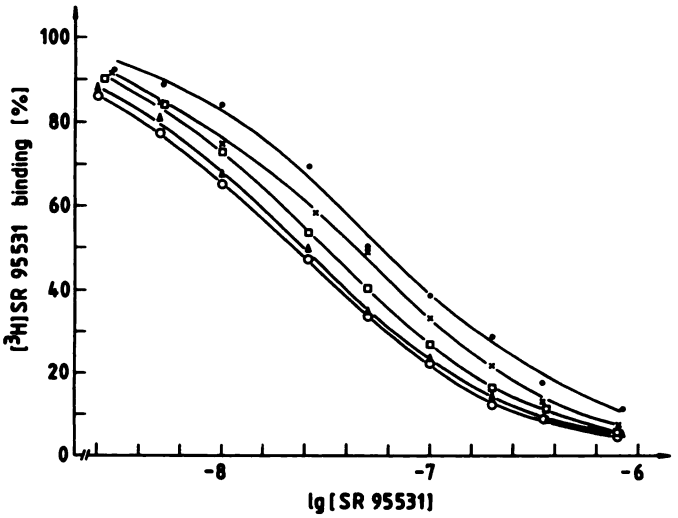

Fig. 1. Temperature dependence of the homologous displacement of [^{3}H]SR 95531 binding. Membrane suspensions were incubated with 3 nM [^{3}H]SR 95531 and different concentrations of SR 95531 in 50 mM Tris-citrate buffer at 0° (○), 10° (△), 19° (□), 28° (×), and 37° (●).

TABLE 1

Saturation analysis of [^{3}H]SR 95531 binding

Synaptosomal membranes were incubated with 3 nM [^{3}H]SR 95531, diluted with seven concentrations of SR 95531 up to 803 nM. Simultaneous incubations were performed at five temperatures. Nonlinear regression of the binding data by LIGAND (18) resulted in the dissociation constants and the maximal numbers of high and low affinity sites. Data are mean ± standard deviation of four experiments.

Temperature	High affinity		Low affinity	
	K_{d1}	B_{max1}	K_{d2}	B_{max2}
	nM	pmol/mg	nM	pmol/mg
0°	8 ± 2	2.5 ± 0.6	163 ± 88	7.6 ± 1.5
10°	14 ± 6	2.0 ± 1.1	129 ± 50	7.4 ± 0.5
19°	16 ± 3	3.1 ± 0.4	186 ± 50	8.4 ± 1.8
28°	23 ± 6	3.3 ± 1.1	314 ± 121	8.5 ± 2.8
37°	27 ± 4	3.1 ± 0.6	413 ± 155	13.2 ± 1.7

Preliminary studies of the displacing potencies of $GABA_A$ agonists and antagonists were performed at 0° and 37° (Table 2). The IC_{50} values of most agonists and antagonists showed significant, but opposite, temperature-dependent shifts, as expressed by the ratios of the IC_{50} values at 37° versus 0° in Table 2. However, the IC_{50} ratios of 4-PIOL and bicuculline methiodide were not significantly different from unity (Table 2). The detailed temperature dependence of equilibrium binding between 0° and 37° (Fig. 2) revealed a flat optimum around 10° for the binding affinities of bicuculline methiodide and R 5135 in each experiment. However, polynomial regression of their temperature dependence (19) was not significantly superior to the linear fit, due to the small number of temperature points. Because van't Hoff plots of the other antagonists (SR 95531, SR 95103, and pitrazepin) were linear (Fig. 2), the nonlinearity cannot be attributed to a temperature-dependent conformational change of the $GABA_A$ receptors or a change in membrane fluidity. Close to physiological temperatures (19–37°) receptor binding of all antagonists was associated with van't Hoff plots with positive slopes (Fig. 2), i.e., negative enthalpy changes

TABLE 2

Displacing potencies of $GABA_A$ agonists and antagonists for the specific binding of [^{3}H]SR 95531 at 37° and 0°

Specific binding of 1.8 nM [^{3}H]SR 95531 at 37° was 46 ± 5% of that at 0° (425 ± 75 fmol/mg of protein). Data are mean ± standard error of the number of experiments in parentheses.

$GABA_A$ ligands	IC_{50}		IC_{50} at 37°
	37°	0°	IC_{50} at 0°
	nM		
Agonists			
Muscimol (7)	164 ± 19	299 ± 40	0.55 ± 0.07[a]
GABA (9)	539 ± 52	988 ± 123	0.55 ± 0.04[a]
Isoguvacine (6)	1800 ± 300	5100 ± 1300	0.35 ± 0.04[a]
THIP (8)	9800 ± 1300	13800 ± 1100	0.71 ± 0.06[b]
Imidazole-4-acetic acid (6)	9800 ± 600	14200 ± 1200	0.69 ± 0.06[b]
4-PIOL (4)	8900 ± 500	9900 ± 900	0.91 ± 0.08
Antagonists			
Pitrazepin (7)	515 ± 51 nM	122 ± 20 nM	4.2 ± 0.6[b]
SR 95531 (8)	67 ± 4 nM	22 ± 2 nM	3.0 ± 0.2[a]
SR 95103 (5)	1800 ± 300	1100 ± 200	1.6 ± 0.1[b]
R 5135 (8)	9.6 ± 1.0 nM	6.2 ± 0.4 nM	1.5 ± 0.1[b]
Bicuculline methiodide (7)	7700 ± 1100	8000 ± 1000	1.0 ± 0.2

[a] $p < 0.001$, significantly different from unity.
[b] $p < 0.005$.

TABLE 3

Thermodynamic parameters of $GABA_A$ agonists and antagonists at the high affinity binding sites for SR 95531

The $\Delta H°$ values were determined from the slope values ($\Delta H°/R$) of the van't Hoff plots in Figs. 1 and 2. The $\Delta G°$ values were calculated from the IC_{50} values of Table 2 at the 37°, according to eqs. 1 and 2. The entropy change ($\Delta S°$) at 37° was calculated as $\Delta S° = -(\Delta G° - \Delta H°)/310$. For R 5135 and bicuculline methiodide, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ values were determined from eqs. 4–7, at 37° (19). Heat capacity changes ($\Delta C_p°$) at 37° were −0.74 kJ/mol·deg for R 5135 and −0.73 kJ/mol·deg for bicuculline methiodide.

$GABA_A$ ligands	$\Delta H°$	$\Delta G°$	$\Delta S°$
	kJ/mol	kJ/mol	J/mol·deg
Agonists			
Muscimol	9.6 ± 1.8	−40.4 ± 0.3	161
Isoguvacine	14.3 ± 1.7	−34.3 ± 0.4	157
GABA	6.7 ± 0.8	−37.4 ± 0.2	142
Imidazole-4-acetic acid	4.6 ± 0.5	−29.9 ± 0.2	111
THIP	4.9 ± 1.1	−29.9 ± 0.3	112
4-PIOL	−0.7 ± 0.8	−30.1 ± 0.1	95
Antagonists			
Pitrazepin	−23.1 ± 2.4	−37.5 ± 0.2	46
SR 95531	−20.6 ± 0.6	−42.7 ± 0.1	71
SR 95103	−12.2 ± 1.3	−34.3 ± 0.4	71
Bicuculline methiodide	−15.1 ± 0.8[a]	−30.4 ± 0.3	49[a]
	−23.2[b]		21[b]
R 5135	−15.8 ± 0.3[a]	−47.8 ± 0.2	103[a]
	−22.3[b]		85[b]

[a] Linear regression.
[b] Polynomial regression via eqs. 4–6.

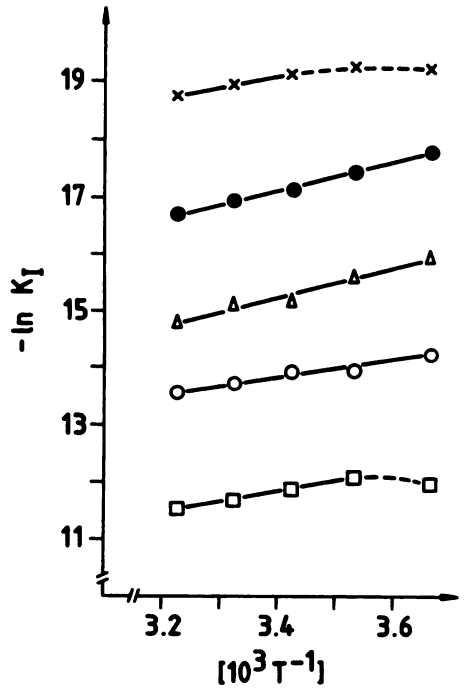

Fig. 2. Van't Hoff plot for the binding of $GABA_A$ antagonists to high affinity sites for SR 95531. ×, R 5135; ●, SR 95531; △, pitrazepin; ○, SR 95103; □, bicuculline methiodide. Data are mean of three to five experiments. K_I values were calculated from IC_{50} values according to eq. 1, with $-\ln K_I = \ln K_e$. Standard errors of the points were <3%. *Dashed lines*, deviations from linearity for bicuculline methiodide and R 5135. Although the small number of temperature points did not enable a significant improvement of the fit by polynomial regression in the *F* test, each experiment showed a similar optimum around 10° for the displacing potencies of bicuculline methiodide and R 5135.

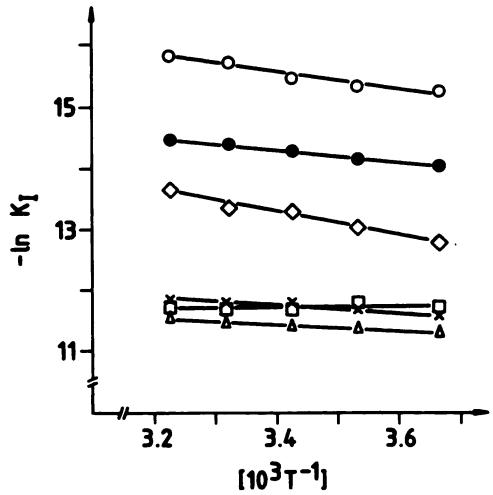

Fig. 3. Van't Hoff plot for the binding of $GABA_A$ agonists to high affinity sites of SR 95531. ○, Muscimol; ●, GABA; ◇, isoguvacine; ×, THIP; □, 4-PIOL; △, imidazole-4-acetic acid.

(Table 3), representing exothermic binding interactions. Polynomial analysis of the temperature dependence of binding for R 5135 and bicuculline methiodide resulted in negative heat capacity changes (Table 3), suggesting hydrophobic binding interactions (20). Due to the temperature dependence of $\Delta H°$, at sufficiently low temperatures this type of interaction becomes endothermic (20). This may explain the temperature dependence previously reported for [^{3}H]bicuculline methiodide binding (10).

Van't Hoff plots for all $GABA_A$ agonists were linear (Fig. 3). The declining slopes in Fig. 3, i.e., the positive enthalpy changes, correspond to endothermic binding interactions for the agonists. This explains why sodium-independent [^{3}H]GABA binding increased with increasing temperature up to about 15° (9). With further increasing temperatures the decrease in [^{3}H]GABA binding (9) might be attributed to accelerated dissociation during the separation of bound [^{3}H]GABA. The van't Hoff plot for 4-PIOL, a partial $GABA_A$ agonist (21), was horizontal (Fig. 3), with no significant enthalpy changes in binding (Table 3).

The van't Hoff plot of the K_{d1} values for the high affinity binding of SR 95531 (Table 1) resulted in a $\Delta H°$ value of −22.5

± 2.4 kJ/mol, which is not significantly different from the $\Delta H°$ value calculated from the K_i values (Table 3). The $\Delta S°$ value of SR 95531 binding was 71 J/mol·K°, using either the K_{d1} values or the K_i values (Table 3). This supports the view that the K_i values can be reliably used to determine the thermodynamic parameters of the binding of GABAergic agents to the high affinity SR 95531 sites. The K_{d2} values for the low affinity binding of SR 95531 showed greater variations (Table 1) and less conclusive temperature dependence. The linear van't Hoff plot of these K_{d2} values resulted in a $\Delta H°$ value of −20.4 ± 6.4 kJ/mol, not significantly different from that for the high affinity binding of SR 95531.

Table 3 shows the Gibbs free energy ($\Delta G°$) values for binding at 37° determined from the cumulative IC_{50} data of Table 2 via the K_{d1} values of Table 1. $\Delta G°$ values were separated into enthalpic and entropic terms. The entropic term ($-T\Delta S°$) is plotted against the enthalpic term ($\Delta H°$) in Fig. 4. The ordinate and abscissa values express the direct contributions of the entropic and enthalpic terms, respectively, to the Gibbs free energy changes, i.e., the driving forces of binding at physiological temperature. Binding of the antagonists at 37° was driven by comparable changes of the enthalpic and entropic terms. Polynomial separation of the thermodynamic parameters for bicuculline methiodide and R 5135 apparently reinforces the contribution of the enthalpic component of their binding (see Fig. 4, *dashed arrows*). In contrast, agonist binding was driven only by entropy increases. The binding of the full $GABA_A$ agonists muscimol and isoguvacine was associated with the greatest entropy changes. The partial agonists 4-PIOL, imidazole-4-acetic acid, and THIP were characterized by entropy changes smaller than that for GABA (Fig. 4).

THIP and imidazole-4-acetic acid resulted in a smaller maximal enhancement of [^{3}H]diazepam binding (22, 23) and a smaller maximal stimulation of $^{36}Cl^-$ influx (24) than did full $GABA_A$ agonists. Muscimol-enhanced [^{3}H]diazepam binding was partially antagonized by these agents (22, 23). In oocytes expressing both brain- and retina (ρ)-type GABA receptors, the maximal chloride currents elicited by THIP (25) and by imidazole-4-acetic acid (26) were smaller than those elicited by GABA. These results support the view that THIP and imidazole-4-acetic acid are partial agonists at several $GABA_A$ receptor subtypes. However, the efficacy of THIP was close to that of GABA with the subunit combination $\alpha5\beta1$ (26). With cultured hippocampal neurons the chloride currents elicited by 4-PIOL were completely blocked by bicuculline methobromide, whereas the currents for the full agonist isoguvacine were partially antagonized by 4-PIOL (21), clearly indicating it to be a partial $GABA_A$ agonist. In conclusion, 4-PIOL, THIP, and imidazole-4-acetic acid can be considered as partial $GABA_A$ agonists in various electrophysiological tests as well as in allosteric interactions with the benzodiazepine binding sites. Because these compounds are situated between the agonists and antagonists in Fig. 4, there is a qualitative correlation between the thermodynamic parameters of binding and the efficacies of $GABA_A$ receptor agonists/antagonists.

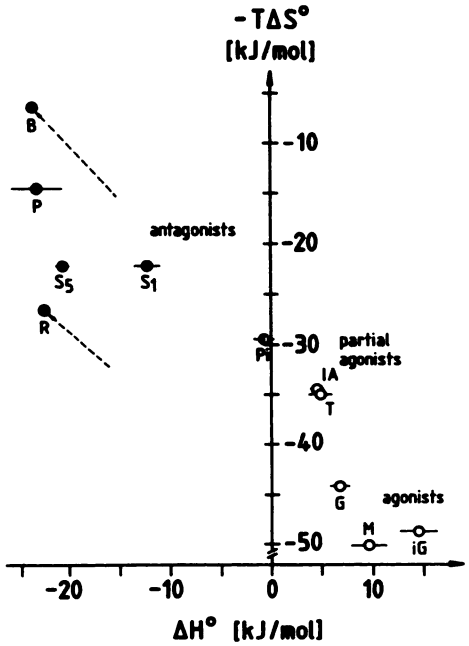

Fig. 4. Plot of the entropic term ($-T\Delta H°$) versus the enthalpic term ($\Delta H°$) for the binding of $GABA_A$ agonists (○) and antagonists (●) to the high affinity sites for SR 95531. The data originate from Table 3 at $T = 310°K$. *P*, pitrazepin; *B*, bicuculline methiodide; *R*, R 5135; S_5, SR 95531; S_1, SR 95103; *Pi*, 4-PIOL; *IA*, imidazole-4-acetic acid; *T*, THIP; *G*, GABA; *M*, muscimol; *iG*, isoguvacine. *Dashed arrows* for bicuculline methiodide and R 5135 start from the parameter values determined by linear regression and end at the values determined by polynomial regression.

The correlation in Fig. 4 is obscured by individual structural differences of the ligands for the $GABA_A$ sites. Large variations in the entropic term for the antagonists in Fig. 4 might be attributed to the various ring structures of the compounds. A major source of the entropy gains of binding is the release of the hydrate shell from the contact areas. This is especially true for hydrophobic binding interactions (19) such as for R 5135. Binding of the large steroidal ring system of R 5135 was associated with the greatest increase in entropy among antagonists, probably due to extensive dehydration upon its binding. On the other hand, entropy changes for the binding of the two phenyl-pyridazinyl-GABA derivatives were identical (Table 3). The 50-fold differences in the affinities of the SR compounds (Table 1) can be entirely attributed to differences in $\Delta H°$ values (Table 3); the ring substituents of SR 95531 contribute to its stronger, more exothermic binding.

Fig. 4 shows a specific manifestation of the general principle of enthalpy-entropy compensation (4, 19, 27). Because all known $GABA_A$ agonists have zwitterionic structures, it is reasonable to expect that any electrostatic interactions with complementary polar residues of the recognition site would lead to a decrease in enthalpy upon binding (4). The enthalpic gains of these possible interactions must have been overcompensated by an endothermic process, because the overall change of enthalpy was positive for $GABA_A$ agonists. This unfavorable balance of enthalpic changes was again overcompensated by a great increase in entropy for $GABA_A$ agonists. The entropic gains for three full $GABA_A$ agonists (about 150 J/mol·K°) are greater than expected from simple electrostatic interactions (28). These anomalies and the correlation between thermodynamic parameters and efficacies suggest a contribution of a conformational change of $GABA_A$ receptors that is relevant in the function of the attached ionophore. It can be speculated that the entropic gains of agonist binding indicate a "relaxation" of the $GABA_A$ receptor complex to a less constrained (open) conformation of the ionophore, and the increase in enthalpy might be attributed to "loosening" of some noncovalent interactions of the ionophore that do not occur upon binding of the antagonists. High affinity binding of the antagonist [^{3}H]SR 95531 is displaced by the agonists with low

affinity, possibly via this conformational change of the receptor complex. There are several lines of evidences that might support this suggestion. 1) Equilibrium studies of receptor binding have revealed that the antagonists bicuculline [^{3}H]methochloride (12) and [^{3}H]SR 95531 (13, 14) bind preferentially to a low affinity population of $GABA_A$ sites. 2) The concept of interconvertible agonist and antagonist conformations of the $GABA_A$ receptor is widely accepted (10–12). 3) Kinetic studies of binding have been very useful in revealing transitions between agonist and antagonist conformations of $GABA_A$ receptors. Pretreatment of the receptors with bicuculline decelerated the on and off rates of [^{3}H]GABA binding (29). Bicuculline preferentially displaced the rapidly dissociating, low affinity binding of [^{3}H]GABA (12), whereas GABA displayed low affinity (micromolar IC_{50}) for the slowly dissociating, high affinity population of [^{3}H]SR 95531 binding sites (13). 4) Dissociation studies suggested negative kinetic cooperativity between $GABA_A$ sites. The addition of excess nonlabeled ligands for the $GABA_A$ sites accelerated the dissociation of [^{3}H]muscimol (30) and [^{3}H]SR 95531 (31). Moreover, addition of excess nonlabeled ligands that were functionally antagonistic to the radioligands resulted in preferential acceleration of dissociation (31). 5) Entropy gains for agonist binding to glycine receptors (5), another member of the family of receptor-regulated ionophores, are similar to those for $GABA_A$ agonists shown in Table 3. The entropy gains for glycine receptor agonist binding have also suggested a less restricted (open) conformation of the glycine receptor-ionophore complex (5).

The correlation in Fig. 4 does suggest a major role for the thermodynamic parameters of binding in the conformational change associated with the function of the $GABA_A$ receptor-ionophore complex, in spite of 1) the heterogeneity of $GABA_A$ receptors, 2) the structural diversity of $GABA_A$ ligands, and 3) the complex transition from ligand binding to receptor function. Because of these complexities, thermodynamic parameters of receptor binding may not correlate with ligand efficacies (4). The correlations for β-adrenergic receptors (2, 3) are related to the family of seven-transmembrane domain, G protein-coupled receptors. Correlation of the type in Fig. 4, together with the thermodynamic data for glycine receptors (5), might have general significance within the family of ligand-gated ionophores. In conclusion, the correlation in Fig. 4 confirms the reliability of [^{3}H]SR 95531 binding to label functionally relevant $GABA_A$ sites and suggests a thermodynamically controlled conformational change of the $GABA_A$ receptor-ionophore complex upon binding to its GABA recognition site.

Acknowledgments

Excellent technical assistance by Mrs. Éva Töry is acknowledged. Critical remarks on this manuscript from Dr. Miklós Simonyi are appreciated.

References

1. De Lorey, T. M., and R. W. Olsen. γ-Aminobutyric acid$_A$ receptor structure and function. *J. Biol. Chem.* **267**:16747–16750 (1992).
2. Weiland, G. A., K. P. Minneman, and P. B. Molinoff. Fundamental difference between the molecular interactions of agonists and antagonists with the β-adrenergic receptor. *Nature (Lond.)* **281**:114–117 (1979).
3. Contreras, M. L., B. B. Wolfe, and P. B. Molinoff. Thermodynamic properties of agonist interactions with the *beta* adrenergic receptor-coupled adenylate cyclase system. I. High- and low-affinity states of agonist binding to membrane-bound adrenergic receptors. *J. Pharmacol. Exp. Ther.* **237**:154–164 (1986).
4. Testa, B., P. Jenner, G. J. Kilpatrick, N. El Tayar, H. Van De Waterbeemd, and C. D. Marsden. Do thermodynamic studies provide information on both the binding to and the activation of dopaminergic and other receptors? *Biochem. Pharmacol.* **36**:4041–4046 (1987).
5. Ruiz-Gomez, A., M. Garcia-Calvo, J. Vázquez, J. C. G. Marvizon, F. Valdivieso, and F. Mayor. Thermodynamics of agonist and antagonist interaction with the strychnine-sensitive glycine receptor. *J. Neurochem.* **52**:1775–1780 (1989).
6. Möhler, H., and T. Okada. Agonist and antagonist benzodiazepine receptor interaction *in vitro*. *Nature (Lond.)* **294**:763–765 (1981).
7. Quast, U., H. Mählmann, and K. O. Vollmer. Temperature- dependence of the benzodiazepine receptor interaction. *Mol. Pharmacol.* **22**:20–25 (1982).
8. Doble, A. Comparative thermodynamics of benzodiazepine receptor ligand interactions in rat neuronal membranes. *J. Neurochem.* **40**:1605–1612 (1983).
9. Enna, S. J., and S. H Snyder. Properties of γ-aminobutyric acid (GABA) receptor binding in rat brain synaptic membrane fraction. *Brain Res.* **100**:81–97 (1975).
10. Möhler, H., and T. Okada. Properties of γ-aminobutyric acid receptor binding with (+)[^{3}H]bicuculline methiodide in rat cerebellum. *Mol. Pharmacol.* **14**:256–265 (1978).
11. Heaulme, M., J. P. Chambon, R. Leyris, C. G. Wermuth, and K. Biziere. Characterization of the binding of [^{3}H]SR 95531, a $GABA_A$ antagonist, to rat brain membranes. *J. Neurochem.* **48**:1677–1686 (1987).
12. Olsen, R. W., and A. M. Snowman. [^{3}H]Bicuculline methochloride binding to low affinity γ-aminobutyric acid receptor sites. *J. Neurochem.* **41**:1653–1663 (1983).
13. Maksay, G. $GABA_A$ receptor populations bind agonists and antagonists differentially and with opposite affinities. *J. Neurochem.* **50**:1865–1871 (1988).
14. McCabe, R. T., J. K. Wamsley, J. P. Yezuita, and R. W. Olsen. A novel $GABA_A$ antagonist, [^{3}H]SR 95531: microscopic analysis of binding in the rat brain and allosteric modulation by several benzodiazepine and barbiturate receptor ligands. *Synapse* **2**:163–173 (1988).
15. Bristow, D. R., and I. L. Martin. Light microscopic autoradiographic localisation in rat brain of the binding sites for the $GABA_A$ receptor antagonist [^{3}H]SR 95531: comparison with the [^{3}H]GABA receptor distribution. *Eur. J. Pharmacol.* **148**:283–288 (1988).
16. Bureau, M. H., and R. W. Olsen. $GABA_A$ receptor subtypes: ligand binding heterogeneity demonstrated by photoaffinity labeling and autoradiography. *J. Neurochem.* **61**:1479–1491 (1993).
17. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
18. Munson, P. J., and P. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand binding systems. *Anal. Biochem.* **107**:220–239 (1980).
19. Edelhoch, H., and J. C. Osborne. The thermodynamic basis of the stability of proteins, nucleic acids, and membranes. *Adv. Protein Chem.* **30**:183–250 (1976).
20. Waelbroeck, M., E. Van Obberghen, and P. De Meyts. Thermodynamics of the interaction of insulin with its receptor. *J. Biol. Chem.* **254**:7736–7740 (1979).
21. Kristiansen, U., J. D. C. Lambert, E. Falch, and P. Krogsgaard- Larsen. Electrophysiological studies of the $GABA_A$ receptor ligand, 4-PIOL, on cultured hippocampal neurones. *Br. J. Pharmacol.* **104**:85–90 (1991).
22. Wong, E. H. F., and L. L. Iversen. Modulation of [^{3}H]diazepam binding in rat cortical membranes by $GABA_A$ agonists. *J. Neurochem.* **44**:1162–1167 (1985).
23. Braestrup, C., M. Nielsen, P. Krogsgaard-Larsen, and E. Falch. Partial agonists for brain GABA/benzodiazepine receptor complex. *Nature (Lond.)* **280**:331–333 (1979).
24. Luu, M. D., A. L. Morrow, S. M. Paul, and R. D. Schwartz. Characterization of $GABA_A$ receptor-modulated ^{36}chloride uptake in rat brain synaptosomes. *Life Sci.* **41**:1277–1287 (1987).
25. Woodward, G. A., L. Polenzani, and R. Miledi. Characterization of bicuculline/baclofen-insensitive (ρ-like) γ-aminobutyric acid receptors expressed in *Xenopus* oocytes. II. Pharmacology of γ-aminobutyric acid$_A$ and γ-aminobutyric acid$_B$ receptor agonists and antagonists. *Mol. Pharmacol.* **43**:609–625 (1993).
26. Kusama, T., C. E. Spivak, P. Whiting, V. L. Dawson, J. C. Schaeffer, and G. R. Uhl. Pharmacology of GABA ρ1 and GABA α/β receptors expressed in *Xenopus* oocytes and COS cells. *Br. J. Pharmacol.* **109**:200–206 (1993).
27. Borea, J. A., K. Varani, L. Guerra, P. Gilli, and G. Gilli. Binding thermodynamics of A_1 adenosine receptor ligands. *Mol. Neuropharmacol.* **2**:273–281 (1992).
28. Westley, J. *Enzymatic Catalysis*. Harper and Row, New York (1969).
29. Maksay, G., and M. K. Ticku. Kinetic differences in GABA receptor binding following GABA and bicuculline methiodide pretreatment. *Neurosci. Lett.* **51**:219–224 (1984).
30. Yang, Y. S. J., and R. W. Olsen. GABA receptor binding in fresh mouse brain membranes at 22°C: ligand-induced changes in affinity. *Mol. Pharmacol.* **32**:266–277 (1987).
31. Maksay, G. Dissociation of muscimol, SR 95531 and strychnine from $GABA_A$ and glycine receptors, respectively, suggests similar cooperative interactions. *J. Neurochem.* **54**:1961–1966 (1990).

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