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Entropy as the predominant driving force of binding to human recombinant $\alpha_x\beta_3\gamma_2$ $GABA_A$ receptors

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Abstract

In order to study the correlation of the thermodynamic driving forces of binding with the efficacies of displacing ligands, the specific binding of [^{3}H]SR 95531 [2-(3-carboxypropyl)3-amino-6-*p*-methoxyphenylpyridazinium bromide], a $GABA_A$ receptor antagonist, was studied in cell lines stably expressing human $\alpha_1\beta_3\gamma_2$ and $\alpha_2\beta_3\gamma_2$ $GABA_A$ receptors. Displacing potencies for the agonists with different efficacies (muscimol, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) and piperidine-4-sulfonic acid) and for antagonists (SR 95531 and 5-(4-piperidyl)isothiazol-3-ol) were determined at 0°C, 20°C and 37°C. Displacing potencies were temperature-nearly independent for $\alpha_1\beta_3\gamma_2$ receptors. At $\alpha_2\beta_3\gamma_2$, receptor binding of the antagonists was exothermic, endothermic for the agonists THIP and piperidine-4-sulfonic acid and isothermic for muscimol. The free energy increments of displacement for the binding of the antagonist [^{3}H]SR 95531 versus the agonist [^{3}H]muscimol approach saturation as a function of the efficacies of the displacers only for $\alpha_1\beta_3\gamma_2$ receptors. This suggests that, for binding to $\alpha_1\beta_3\gamma_2$ $GABA_A$ receptors, displacement is an efficacy-dependent interaction predominantly driven by entropic increases.

Keywords: $GABA_A$ receptors, Recombinant; Thermodynamics; $GABA_A$ receptor antagonist; $GABA_A$ receptor agonist; Entropy change

1. Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in mammalian brain. $GABA_A$ receptors belong to the superfamily of transmitter-gated ion channels (Barnard, 1996). Several $GABA_A$ receptor subunits have been identified including α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε and π subunits (Whiting et al., 1999). Five subunits form chloride channels. Since α and β subunits exert predominant influence upon the efficacies and potencies of $GABA_A$ agonists, GABA binding sites are thought to reside at the interface of these subunits (Ebert et al., 1994). Heterocyclic derivatives of GABA cover the whole continuum of efficacies between antagonists and full agonists (Krogsgaard-Larsen et al., 1997).

The pharmacological importance of modulating GABAergic neurotransmission has stimulated the study of receptor binding interactions, in order to find correlations with the pharmacological properties of GABAergic ligands. Allosteric binding interactions and the kinetics of binding have revealed several such correlations (e.g. Maksay, 1996a). The thermodynamics of binding has shown a unique correlation with the efficacies of a series of $GABA_A$ receptor partial and full agonists and antagonists (Maksay, 1994). The binding of agonists was driven entirely by entropic increases, whereas binding of antagonists was driven mainly by enthalpic decreases. Moreover, thiocyanate, a chaotropic agent, selectively enhanced the entropic gains for the binding of the antagonist bicuculline to suggest "entropic trapping" of bicuculline in a sterically constrained binding pocket (Maksay, 1998). These thermodynamic analyses have been based on the different temperature dependencies of the displacing potencies for the binding of the antagonist [^{3}H]SR 95531 [2-(3-carboxypropyl)3-amino-6-*p*-methoxyphenylpyridazinium bromide] to homogenates of native $GABA_A$ receptors from rat whole brain.

In order to obtain more specific knowledge about the subunit dependent properties, recombinant $GABA_A$ receptors in cell cultures can be used for binding studies, while ionophore function can be examined via electrophysiological methods in frog oocytes. Different α subunits affected

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the transduction mechanism and ionophore function of $GABA_A$ receptors rather than binding per se (Ebert et al., 1997). Nevertheless, $\alpha_x\beta_3\gamma_2$ $GABA_A$ receptors with different α subunits have shown opposite rank orders of binding affinities for $GABA_A$ receptor agonists versus antagonists (Ebert et al., 1997).

We use here recombinant $GABA_A$ receptors with different α subunits to confirm the correlation between the thermodynamic driving forces of binding and the efficacies of ligands and extend it from structural changes of the ligands to those of the receptor subunits.

2. Materials and methods

2.1. Materials

[^{3}H]SR 95531 (49 Ci/mmol) was obtained from New England Nuclear, piperidine-4-sulfonic acid (P4S) and THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) from Tocris–Cookson. Thio-4-PIOL [5-(4-piperidyl)isothiazol-3-ol] was a gift from Dr. Bente Frolund (Copenhagen, Denmark). SR 95531 was from SANOFI (France).

2.2. Binding studies

Mouse fibroblast L(tk$^-$) cell lines stably expressing human $\alpha_x\beta_3\gamma_2$ $GABA_A$ receptors were grown and harvested as described previously (Hadingham et al., 1992). Cells were homogenized, washed by centrifugation in Tris-buffered saline and kept frozen (Hadingham et al., 1992). Frozen membranes were thawed, resuspended in 50 mM Tris citrate buffer (pH 7.1), centrifuged at $20{,}000 \times g$ for 20 min and refrozen. Before the binding assay, the suspension was similarly thawed and centrifuged. One mililiter samples containing about 0.2 mg protein were incubated with 1.6 nM [^{3}H]SR 95531 in 50 mM Tris citrate buffer (pH 7.1) for 15–50 min (between 37°C and 0°C, respectively). Duplicate aliquots were filtered under vacuum on Whatman GF/B filters and washed twice with 3 ml of ice-cold buffer. Nonspecific binding was determined in the presence of 1 mM GABA.

2.3. Data analysis

The 50% displacing concentrations (IC_{50} values) of the compounds were determined via fitting to the displacement curves. Inhibition constants (K_i values) of SR 95531 and other displacers were calculated from the IC_{50} values at two to three temperatures according to the Cheng–Prusoff equation: $K_i = IC_{50}/(1 + c/K_D)$ where c is the concentration of [^{3}H]SR 95531 and K_D is its dissociation constant. K_D values of SR 95531 were replaced by K_i values. Enthalpic (ΔH) and entropic changes (ΔS) of binding were determined as described previously (Maksay, 1994). Briefly, Gibbs free energy changes were calculated at 37°C via $\Delta G = RT \ln K_i$. Linear van't Hoff plots ($\ln K_i$ versus $1/T$) result in slope values of $\Delta H/R$. Entropy changes of binding were determined from $\Delta G = \Delta H - T\Delta S$. To compare displacement data for agonist versus antagonist binding, K_i values for [^{3}H]SR 95531 binding ($K_{i,\,antag}$) were divided by the K_i values for [^{3}H]muscimol binding ($K_{i,agon}$) determined at 20°C (Ebert et al., 1997). For the temperature-nearly independent binding to $\alpha_1\beta_3\gamma_2$ $GABA_A$ receptors, means of $K_{i,antag}$ values at 0°C and 37°C were calculated. The free energy increments of binding ($\Delta\Delta G$) to displace antagonist versus agonist radioligands were determined from the K_i ratios: $\Delta\Delta G = RT \ln K_{i,\,antag}/K_{i,\,agon}$. The use of phosphate instead of Tris buffer for [^{3}H]muscimol binding (Ebert et al., 1997) might cause a constant shift in $\Delta\Delta G$, but could not possibly result in efficacy-dependent differences.

3. Results

We used membrane preparations of cell cultures expressing $\alpha_x\beta_3\gamma_2$ $GABA_A$ receptors and compared their suitability for specific [^{3}H]SR 95531 binding. $\alpha_2\beta_3\gamma_2$ $GABA_A$ receptors displayed the highest ratio of specific versus nonspecific binding. Preliminary saturation analysis of [^{3}H]SR 95531 binding resulted in K_D values of about 10 nM and 1 μM for the high and low affinity components, respectively. For $\alpha_1\beta_3\gamma_2$ $GABA_A$ receptors, non-

Table 1
The parameters of displacement of [^{3}H]SR 95531 binding to human recombinant $\alpha_1\beta_3\gamma_2$ $GABA_A$ receptors by antagonists and agonists at 0°C and 37°C

Compound	IC_{50} (nM)		n_H^a		$K_{i,\,37}/K_{i,\,0}^b$
	0°C	37°C	0°C	37°C	
SR 95531	11 ± 3	14 ± 4	1.08 ± 0.13	1.09 ± 0.08	1.32
Thio-4-PIOL	837 ± 134	996 ± 96	0.91 ± 0.06	086 ± 0.10	1.23
Piperidine-4-sulfonic acid	230 ± 36	207 ± 44	0.98 ± 0.08	1.02 ± 0.10	0.93
THIP	558 ± 103	377 ± 67	1.06 ± 0.15	0.92 ± 0.09	0.70
Muscimol	9.6 ± 1.5	8.2 ± 1.7	1.17 ± 0.15	1.12 ± 0.33	0.89

Synaptosomal membranes of the cell lines stably expressing human $\alpha_1\beta_3\gamma_2$ $GABA_A$ receptors were incubated with 1.6 nM [^{3}H]SR 95531 in 50 mM Tris citrate buffer (pH 7.1). Data are means ± S.E. of four to six experiments.

[a] n_H is the slope factor of displacement.

[b] The ratio of K_i values at 37°C versus 0°C.

specific binding of 1.6 nM [^{3}H]SR 95531 was high (between 20% and 45% at 0°C and 37°C, respectively). It allowed a displacement study, rather than a reliable saturation analysis. Therefore, a displacement study was performed subsequently for α_1 and α_2 subunit containing receptors.

Table 1 contains the parameters of displacement for five compounds at α_1 subunit containing receptors at 0°C and 37°C. The compounds include the antagonists SR 95531 and thio-4-PIOL, the partial agonists piperidine-4-sulfonic acid and THIP as well as the full agonist muscimol. None of the IC_{50} values displayed significant temperature-dependent differences for 0°C and 37°C (Table 1), and IC_{50} values at 20°C did not differ significantly from those at 0°C and 37°C in preliminary experiments (data not shown). The slope factors (n_H) of displacement were not significantly different from unity (Table 1). The ratios of $K_{i,37}$ and $K_{i,0}$ values were also close to unity (Table 1).

Table 2 summarizes the displacing potencies at $\alpha_2\beta_3\gamma_2$ receptors. The IC_{50} value of the antagonist SR 95531 was significantly lower at 0°C than at 37°C. In contrast, the IC_{50} values of the agonists piperidine-4-sulfonic acid and THIP decreased with increasing temperature (Table 2). Of distinctive interest was piperidine-4-sulfonic acid which has low efficacy for $\alpha_1\beta_3\gamma_2$ receptors, while it is a full agonist for $\alpha_2\beta_3\gamma_2$ receptors (see Table 3). Unexpectedly, the temperature-dependent changes in the IC_{50} values for the full agonist muscimol did not reach statistical significance for $\alpha_2\beta_3\gamma_2$ receptors either (Table 2). All slope factors of displacement were close to unity (data not shown).

Fig. 1 shows van't Hoff plots of K_i values of displacement for $\alpha_2\beta_3\gamma_2$ receptors. The positive change in enthalpy upon the endothermic binding of piperidine-4-sulfonic acid can be determined from the slope of its linear van't Hoff plot. The K_i (IC_{50}) values of thio-4-PIOL were not significantly different at 20°C and 0°C, while its K_i value was significantly higher at 37°C (Table 2 and Fig. 1). This discrepancy might reflect the unique dual nature of thio-4-PIOL being a strong antagonist (Ebert et al., 1997) mixed with a low efficacy agonist (Table 3 and Maksay et al., 2000).

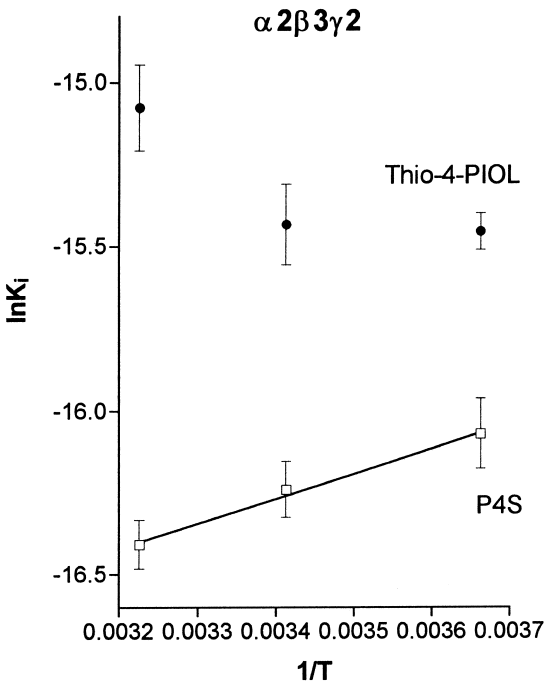

Fig. 1. Van't Hoff plots of the displacement of [^{3}H]SR 95531 binding to recombinant human $\alpha_2\beta_3\gamma_2$ $GABA_A$ receptors by thio-4-PIOL and piperidine-4-sulfonic acid (P4S). Membrane preparations from the cell lines were incubated in 50 mM Tris citrate buffer (pH 7.1) with 1.6 nM [^{3}H]SR 95531 and different concentrations of $GABA_A$ receptor agonists and antagonists for 15–50 min (at 37°C and 0°C, respectively). K_i values were calculated from the 50% displacing concentrations; $1/T$ is expressed in K^{-1}. The points represent means ± S.E. of four to six experiments. K_i values were calculated from IC_{50} values in Table 2.

Table 2
The temperature-dependence of the displacing potencies of $GABA_A$ receptor antagonists and agonists on human recombinant $\alpha_2\beta_3\gamma_2$ receptors

Compound	IC_{50} (nM)			$K_{i,37}/K_{i,0}$[a]
	0°C	20°C	37°C	
SR 95531	4.6 ± 0.7[b]	7.3 ± 1.5	10.4 ± 2.1	2.93
Thio-4-PIOL	299 ± 17	264 ± 32[b]	347 ± 48	1.51
Piperidine-4-sulfonic acid	166 ± 19[b]	116 ± 9	90 ± 7	0.70
THIP	1752 ± 158[b]		1167 ± 99	0.86
Muscimol	15.0 ± 1.5		2.2 ± 1.7	1.05

Synaptosomal membranes of the cell lines stably expressing human $\alpha_2\beta_3\gamma_2$ $GABA_A$ receptors were incubated with 1.6 nM [^{3}H]SR 95531 in 50 mM Tris citrate buffer (pH 7.1). Data are means ± S.E. of four to six experiments.

[a] The ratio of K_i values at 37°C versus 0°C.

[b] $P < 0.05$, significantly different from IC_{50} values at 37°C in an unpaired Student's *t*-test.

Van't Hoff analysis was performed for the displacing agents. It was assumed that their van't Hoff plots are linear. Linearity has been demonstrated for SR 95531, THIP and muscimol with native $GABA_A$ receptors of rat brain (Maksay, 1994) and for piperidine-4-sulfonic acid, here. Since the van't Hoff plot of thio-4-PIOL deviated from linearity for $\alpha_2\beta_3\gamma_2$ receptors, its van't Hoff analysis was restricted for 20–37°C. This semiquantitative method enabled us to estimate the rough contribution of the two components of the thermodynamic driving forces of binding at 310 K. The enthalpic term (ΔH) and the entropic term ($-T\Delta S$) were plotted in Fig. 2. For comparison, Fig. 2 also contains the analagous ΔH and $-T\Delta S$ data on native $GABA_A$ receptors of rat brain (Maksay, 1994). The semiseparated patterns for antagonists, partial and full agonists confirm the previous correlation with efficacies. Of special note is piperidine-4-sulfonic acid, which is a low efficacy partial agonist for $\alpha_1\beta_3\gamma_2$ receptors (P_1 in Fig. 2), while it is a full agonist for $\alpha_2\beta_3\gamma_2$ receptors (P_2 in Fig. 2). The displacing potencies of these

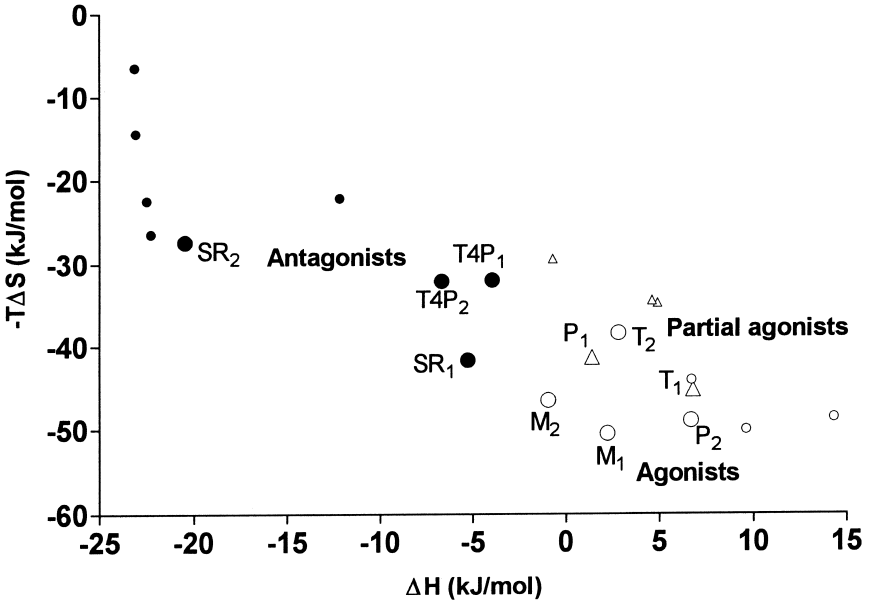

Fig. 2. Plot of the entropic term ($-T\Delta S$) versus the enthalpic term (ΔH) for the binding of $GABA_A$ receptor antagonists (●), partial (△) and full (○) agonists to recombinant human $\alpha_x\beta_3\gamma_2$ $GABA_A$ receptors. $T\Delta S$ was expressed for $T = 310$ K. Compound codes: SR 95531 (SR), thio-4-PIOL (T4P), piperidine-4-sulfonic acid (P), THIP (T) and muscimol (M). Their indices, 1 and 2, are related to α_1 and α_2 subunit-containing receptors, respectively. Similar data for the binding of $GABA_A$ receptor antagonists, partial and full agonists to native $GABA_A$ receptors of rat whole brain are included in the plot for comparison as taken from Maksay (1994). They are indicated by smaller similar symbols.

compounds for $\alpha_x\beta_3\gamma_2$ receptors have been determined previously on the specific binding of the agonist [^{3}H]muscimol at 20°C (Ebert et al., 1997). In order to compare displacement data for agonist versus antagonist binding, K_i values for [^{3}H]SR 95531 binding ($K_{i,antag}$) were divided by K_i values for [^{3}H]muscimol binding ($K_{i,\,agon}$). Table 3 contains $K_{i,\,antag}/K_{i,\,agon}$ ratios together with the efficacies of the compounds. It is remarkable that the rank order of the K_i ratios is identical with that of the efficacies for $\alpha_1\beta_3\gamma_2$ receptors. The free energy increments of binding ($\Delta\Delta G$) to displace antagonist versus agonist radioligands were determined from the K_i ratios in Table 3, and plotted as a function of the efficacies in Fig. 3. $\Delta\Delta G$ values approach saturation for $\alpha_1\beta_3\gamma_2$ receptors, while there is no such obvious correlation for $\alpha_2\beta_3\gamma_2$ receptors.

Table 3
The efficacies of GABAergic agents and the ratios of the K_i values for antagonists versus agonist radioligands

Compounds	Efficacies[a]		$K^b_{i,\,antag}/K^c_{i,\,agon}$	
	$\alpha_1\beta_3\gamma_2$	$\alpha_2\beta_3\gamma_2$	$\alpha_1\beta_3\gamma_2$	$\alpha_2\beta_3\gamma_2$
SR 95531	0	0	0.29	0.74
Thio-4-PIOL	5[d]	4[d]	0.47	0.39
Piperidine-4-sulfonic acid	21	96	1.26	0.95
THIP	70	98	1.51	2.88
Muscimol	97	96	1.94	1.26

[a]Taken from Ebert et al. (1997), as determined by voltage-clamp electrophysiology for oocytes.

[b]The mean of K_i values at 0°C and 37°C for [^{3}H]SR 95531 binding for $\alpha_1\beta_3\gamma_2$ receptors (Table 1) and K_i values at 20°C for $\alpha_2\beta_3\gamma_2$ receptors (Table 2).

[c]K_i values for [^{3}H]muscimol binding at 20°C were taken from Ebert et al. (1997).

[d]Determined for the corresponding human $\alpha_x\beta_3\gamma_2$ $GABA_A$ receptors in oocytes of xenopus laevis by voltage-clamp electrophysiology (Maksay et al., 2000).

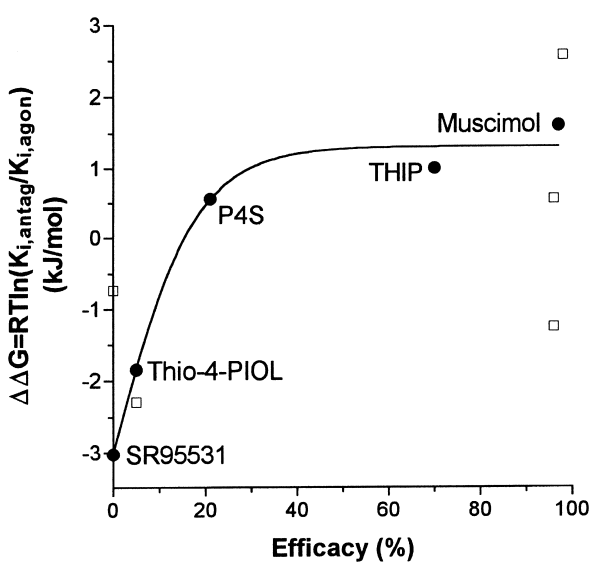

Fig. 3. The correlation of the free energy increments of binding ($\Delta\Delta G$) to displace antagonist versus agonist radioligands with the efficacies of the displacers for $\alpha_1\beta_3\gamma_2$ $GABA_A$ receptors (●). $RT\ln(K_{i,antag}/K_{i,agon})$ was calculated from the ratios $K_{i,antag}/K_{i,agon}$ in Table 3. They correspond to the free energy increments of binding ($\Delta\Delta G$) in displacement of antagonist versus agonist radioligands. $\Delta\Delta G$ values approach saturation as a function of efficacies for $\alpha_1\beta_3\gamma_2$ $GABA_A$ receptors, while there is no such obvious correlation for $\alpha_2\beta_3\gamma_2$ receptors (data labelled with □).

4. Discussion

The suitability of $\alpha_x\beta_3\gamma_2$ receptors in cell lines for [^{3}H]SR 95531 binding depended on the affinities of these recombinant receptors to SR 95531, since the levels of receptor expression were similar. The $\alpha_2\beta_3\gamma_2$ subunit combination was most suitable for [^{3}H]SR 95531 binding. This is in agreement with the greatest displacing potencies of the antagonists for [^{3}H]muscimol binding to $\alpha_2\beta_3\gamma_2$ receptors (Ebert et al., 1997). In contrast to this antagonist-preferring α_2 subunit-containing combination, the agonist-preferring combinations (e.g. with α_5) bound [^{3}H]SR 95531 with lower affinity and with high nonspecific binding.

Data on temperature-dependence of binding to recombinant $GABA_A$ receptors have not been published yet. The temperature sensitivities of recombinant human $\alpha_1\beta_3\gamma_2$ and $\alpha_2\beta_3\gamma_2$ receptors were smaller than those of native $GABA_A$ receptors in rat brain homogenates (Maksay, 1994). Homogenates of native receptors should result in a composite temperature sensitivity according to the relative abundance and affinity of different subunit combinations for [^{3}H]SR 95531. Since $\alpha_1\beta_2\gamma_2$ and $\alpha_2\beta_3\gamma_2$ receptors are predominant in rat brain (Whiting et al., 1999), these might have significantly contributed to the temperature sensitivity reported (Maksay, 1994). The reason for the lower temperature sensitivity of recombinant human $\alpha_x\beta_3\gamma_2$ $GABA_A$ receptors remains to be clarified. Several temperature-nearly independent binding processes including receptor binding of β-adrenergic antagonists have been attributed to hydrophobic, sterically constrained environments (Miklavc, 1996).

SR 95531 binding is heterogeneous and its high affinity component appears to represent the physiologically more relevant low affinity component of $GABA_A$ receptor agonist binding (Maksay, 1988). The nonspecific and low affinity components of SR 95531 binding (especially for α_1 receptors at 37°C) led us to perform displacement instead of saturation analysis. A low concentration of 1.6 nM [^{3}H]SR 95531 was applied, which labels predominantly the high affinity component of its binding sites (Maksay, 1994). This concentration was well below the K_D values of the high and low affinity components of [^{3}H]SR 95531 binding. The slope values of displacement close to unity also support the notion that our displacement data correspond predominantly to a homogeneous population of high affinity binding sites. It was thus assumed that the K_D values of the high affinity binding of [^{3}H]SR 95531 could be replaced by its K_i values to calculate the binding constants of the displacers.

The $K_{i,37}/K_{i,0}$ ratios above unity for SR 95531 and thio-4-PIOL represent exothermic binding interactions such as found for native $GABA_A$ receptors with all antagonists (Maksay, 1994). Higher affinity of SR 95531 to $\alpha_2\beta_3\gamma_2$ versus $\alpha_1\beta_3\gamma_2$ receptors at low temperatures can be explained by its more exothermic interaction at α_2 receptors (Fig. 2). This is in agreement with the general antagonist-preferring impact of the α_2 subunit observed earlier for [^{3}H]muscimol binding (Ebert et al., 1997).

The $K_{i,37}/K_{i,0}$ ratios below unity for piperidine-4-sulfonic acid and THIP on $\alpha_2\beta_3\gamma_2$ receptors represent endothermic interactions. The binding of piperidine-4-sulfonic acid and THIP is driven by great increases in entropy characteristic for agonists with high efficacies. In agreement with piperidine-4-sulfonic acid being a partial agonist at α_1 subunit-containing receptors and a full agonist at α_2 containing ones, P_1 and P_2 are distantly situated in Fig. 2. Preferential affinity of piperidine-4-sulfonic acid to $\alpha_2\beta_3\gamma_2$ receptors at high temperatures can be explained by its more endothermic interaction.

The binding of all full agonists was endothermic at $GABA_A$ receptors of rat brain (Maksay, 1994). Therefore, an important but unexpected finding here, was that the binding of the full agonist muscimol was temperature-nearly independent for both receptors. This suggests that it is probably not the change of enthalpy that is primarily correlated with efficacy, but the great increase in entropy.

It is a general observation that antagonists displace the binding of antagonists preferentially and vice versa (Heaulme et al., 1987). Remarkably, the free energy changes of binding to displace antagonist versus agonist radioligands correlated with the efficacies of the displacers only for $\alpha_1\beta_3\gamma_2$ receptors, but not for $\alpha_2\beta_3\gamma_2$ receptors. The curve in Fig. 3 appears to approach saturation. This suggests an increasingly unfavourable change in free energies upon displacement of the antagonist radioligand by a displacer with increasing agonist efficacy. It is tempting to speculate that the free energy increments of binding are associated with a conformational change of the receptor elicited by agonists. A major difference between $\alpha_2\beta_3\gamma_2$ and $\alpha_1\beta_3\gamma_2$ receptors is the temperature-nearly independent nature of binding to $\alpha_1\beta_3\gamma_2$ receptors. That is, enthalpic changes have minor contributions to the driving force of binding. Thus, $\Delta\Delta G$ representing a transition between antagonist and agonist states of the receptors might predominantly be determined by changes in entropy.

In the light of the entropy–enthalpy compensation, it is difficult to attribute the separation pattern according to efficacies in Fig. 2 predominantly to either of the two thermodynamic parameters of binding. However, the above evidences seem to support the predominant role of the entropic term in binding. The much higher affinities of agonists in binding in contrast to ionophore function may very well reflect desensitized receptors during long incubation in binding studies (Ebert et al., 1997). Yet, displacement by agonists seems to require a conformational transition of the receptor, the extent of which depends on the efficacies of the displacers. The underlying process seems to be predominantly driven by increases in entropy.

In conclusion, α_1 and α_2 subunit-containing $GABA_A$ receptors have revealed different and similar properties as well. The α subunits impart different patterns of potencies

and efficacies to the ligands of $GABA_A$ receptors. The five times broader range of displacing potencies (a ratio of 380 versus 76) for the α_2 subunit at 0°C can be attributed to the greater contribution of the enthalpic term of binding to α_2 versus α_1 subunit-containing $GABA_A$ receptors. The correlation between efficacies and free energy increments of the temperature-nearly independent binding of agonists versus antagonists to α_1 containing $GABA_A$ receptors might be explained with the entropic term. In spite of these differences, α_1 and α_2 subunit-containing $GABA_A$ receptors fit into a common correlation of thermodynamic discrimination of the efficacies. The above correlations between binding parameters and efficacies of the ligands might be attributed to pharmacologically relevant conformational changes of the receptors upon agonist binding predominantly driven by entropic changes.

Putative conformational changes of $GABA_A$ receptors are also suggested by the allosteric interactions of the binding of the channel blocking radioligand [^{35}S]*t*-butylbicyclophosphorothionate (TBPS): barbiturates displace [^{35}S]TBPS binding to native $GABA_A$ receptors of rat brain via acceleration of its dissociation (Maksay et al., 1996). The temperature-independence of the displacing potency of 1-methyl-5-phenyl-5-propyl barbituric acid also supports the predominant role of the entropic factor in binding. Arrhenius analysis revealed that the chloride channel opening R(−) enantiomer of 1-methyl-5-phenyl-5-propyl barbituric acid decreased the activation energy for accelerated displacement of [^{35}S]TBPS binding (Maksay et al., 1996).

The conformational changes in ionophore states might be analogous within the superfamily of transmitter gated ion channels, because correlations similar to Fig. 2 have been found for other members of the superfamily, e.g. glycine (Ruiz-Gomez et al., 1989) and 5-HT_3 type serotonin receptors as well (Borea et al., 1996; Maksay, 1996b, in press).

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