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SHORT COMMUNICATION

Differential Effects of Thiocyanate on the Binding Thermodynamics of Bicuculline Methiodide Versus SR95531 (Gabazine) to the γ-Aminobutyric Acid Receptor-Ionophore Complex

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ABSTRACT. The temperature dependence of the binding of [^{3}H]SR 95531 (Gabazine), an antagonist of γ-aminobutyric acid ($GABA_A$) receptors, was studied in synaptosomal membranes of rat brain in the presence of 50 mM KSCN. The displacing potencies of the antagonists bicuculline methiodide and Gabazine were determined at five temperatures between 0° and 37°. Van't Hoff plots of the displacing potencies were analyzed by linear regression in the presence and absence of thiocyanate. Thiocyanate hardly affected the exothermic ionic binding interaction of gabazine. In contrast, thiocyanate strongly potentiated the binding of bicuculline methiodide and deprived it of its exothermic nature. The enhanced binding of bicuculline methiodide in the presence of chaotropic SCN^- ions might be reconciled with "entropic trapping" in a sterically constrained hydrophobic binding pocket. BIOCHEM PHARMACOL **56**;6:729–731, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. $GABA_A$ receptor binding; thermodynamics of binding; $GABA_A$ antagonists; bicuculline methiodide; Gabazine (SR 95531); chaotropic effects of thiocyanate

A-type receptors of the most important inhibitory neurotransmitter $GABA_A$† belong to the superfamily of neurotransmitter receptor-ionophore complexes. The kinetics and thermodynamics of $GABA_A$-receptor binding have revealed several correlations with ionophore function [1]. The thermodynamic parameters of binding correlated with the efficacies of $GABA_A$ agonists, partial agonists and antagonists [2]. Exothermic binding of $GABA_A$ antagonists was accompanied by small increases in entropy, while endothermic binding of agonists was driven by large increases in entropy [2]. Bicuculline is a classic and selective antagonist of $GABA_A$ receptors and gabazine (SR 95531), a recent pyridazinyl derivative of GABA, is the only suitable radiolabeled antagonist [3] which also enabled the thermodynamic analysis of $GABA_A$-receptor binding to be performed [2]. Monovalent Eccles anions such as chloride and thiocyanate, which penetrate across $GABA_A$-receptor-regulated ion channels [4], affect $GABA_A$-binding sites as well. $GABA_A$ ionophores have greatest permeabilities for thiocyanate ions [5]. Thiocyanate exerted opposite shifts on the binding affinities of $GABA_A$ antagonists versus agonists and suggested the role of hydrophobic accessory sites for antagonists [5]. The affinity of bicuculline was strongly enhanced by thiocyanate [6], while the binding of zwitterionic pyridazinyl $GABA_A$ antagonists such as gabazine was hardly affected [7]. Thiocyanate results in highest enhancements of binding for several radioligands of $GABA_A$, glycine [8] and other receptors. Some anomalic effects of thiocyanate might be associated with its chaotropic activity [9]. Therefore, the effects of thiocyanate were compared here for the binding thermodynamics of a more stable methiodide derivative of bicuculline, BCM vs gabazine.

MATERIALS AND METHODS

Materials

[^{3}H]SR 95531 (49 Ci/mmol) was obtained from New England Nuclear and BCM from Sigma. Gabazine was kindly donated by SANOFI.

Binding Studies

Extensively washed synaptosomal membranes were prepared from whole brains of male Wistar rats as described [2]. Synaptosomal membranes were suspended in 50 mM Tris citrate (pH = 7.1) containing 50 mM KSCN and incubated with 1.7 nM [^{3}H]SR 95531 simultaneously at five temperatures between 0° and 37°. Incubations were terminated after 15 min (37°) to 40 min (0°) by rapid filtration [2]. Nonspecific binding was determined in the presence of 1 mM GABA. All thermodynamic parameters were determined as described previously [2] but were considered as apparent ($\Delta G°'$, $\Delta H°'$ and $\Delta S°'$), because IC_{50} values were

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† *Abbreviations:* BCM, bicuculline methiodide; GABA, γ-aminobutyric acid; and SR 95531, Gabazine.

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TABLE 1. The effect of temperature on the displacing potencies of gabazine and BCM on [^{3}H]SR 95531 binding in the presence of 50 mM KSCN

	IC_{50} (nM)	
t (°)	BCM	Gabazine
37	744 ± 80	153 ± 20
28	828 ± 58	90 ± 3
19	792 ± 111	63 ± 6
10	876 ± 171	82 ± 8
0	1455 ± 302	54 ± 3

Synaptosomal membranes of rat whole brains were incubated with 1.7 nM [^{3}H]SR 95531 in 50 mM Tris citrate (pH = 7.1) in the presence of 50 mM KSCN and different concentrations of the displacers. Data are means ± SEM of 3–6 experiments.

used instead of the dissociation constants of the inhibitors (K_I) in the equation $\Delta G° = -RTlnK_A = RTlnK_I$. This approximation is justified because the concentration of [^{3}H]SR 95531 (1.7 nM) was much lower than the dissociation constant (K_D) of [^{3}H]SR 95531 binding [2].

RESULTS AND DISCUSSION

Table 1 shows the displacing potencies of the $GABA_A$ antagonists BCM and Gabazine on [^{3}H]SR 95531 binding between 0° and 37° in the presence of 50 mM KSCN. These data were plotted in Fig. 1 on a logarithmic scale together with those determined previously under identical conditions in the absence of thiocyanate [2]. Thiocyanate decreased the displacing potencies of gabazine, with a small parallel shift in its van't Hoff plot (Fig. 1). In contrast, the displacing potencies for BCM were strongly enhanced by thiocyanate, its horizontal van't Hoff plot (Fig. 1) representing a temperature-nearly-independent binding process. As the van't Hoff plots for BCM deviated from linearity at

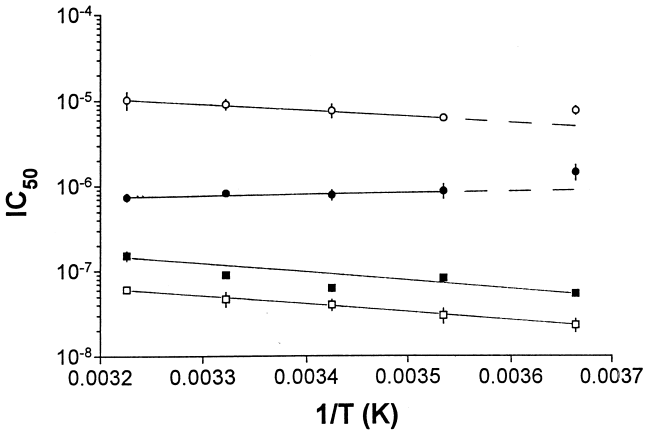

FIG. 1. Van't Hoff plots for the displacing potencies of gabazine (■) and BCM (●) on [^{3}H]SR 95531 binding in the presence of 50 mM KSCN. For comparison, the figure also contains the data for BCM (○) and gabazine (□) determined under identical conditions in the absence of thiocyanate, as taken from Ref. 2. Linear regression resulted in the thermodynamic parameters of binding in Table 2.

TABLE 2. The effects of 50 mM KSCN on the thermodynamic parameters of binding to $GABA_A$ receptors

Displacers	BCM		Gabazine	
Thermodynamic parameters	ΔH°′ kJ/mol	ΔS°′ J/mol°	ΔH°′ kJ/mol	ΔS°′ J/mol°
+50 mM KSCN	3.5 ± 1.8†	129	−16.4 ± 5.9	78
Control*	−15.1 ± 0.8†	49	−20.6 ± 0.6	71

*Taken from Ref. 2.

†Determined from the slopes of the van't Hoff plots in Fig. 1. Because the points at 0° significantly deviated from the line, linear regressions were restricted to 10°–37°.

0° both in the absence and presence of thiocyanate (Fig. 1), these data were omitted from the linear regression. Table 2 contains the apparent changes in enthalpy and entropy upon binding of the antagonists. Thiocyanate did not significantly affect the change in enthalpy upon binding of gabazine (Table 2), with the binding of this zwitterionic GABA-mimetic antagonist remaining exothermic. The entropy increase resulted in an approximate −22 to −24 kJ/mol contribution to the driving force for the binding of gabazine around physiological temperatures, which is slightly greater than the enthalpic term. In contrast, thiocyanate completely eliminated the exothermic driving force for BCM binding, while the entropy increase was more than doubled (Table 2). These temperature-nearly-independent processes have been attributed to "entropic trapping" and relatively nonspecific interactions in hydrophobic, sterically constrained environments [10]. Deviations from linearity of the van't Hoff plots for BCM at low temperature do not seem to reflect a change in receptor-membrane structure, since such deviations were only observed for BCM and the steroidal antagonist R 5135 [2]. The temperature dependencies of ΔS and ΔH with a negative heat capacity change Δc_p° might be reconciled with hydrophobic interactions [11]. The chaotropic effect of thiocyanate ions might facilitate the removal of ordered water molecules from the contact surfaces for BCM binding. Another chaotropic anion, perchlorate, exerted similar effects on the binding of [^{3}H]BCM to $GABA_A$ receptors [12].

In conclusion, the distinct effects of thiocyanate ions on the thermodynamic parameters of receptor binding exaggerate and thus elucidate the importance of entropic factors for BCM binding, while for gabazine the enthalpic term remains equally important. This study illustrates how the correlation between thermodynamic parameters of binding and receptor activation might be obscured [13].

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