# INTERACTION BETWEEN GABA- AND $Cl^-$-IONOPHORE BINDING SITES OF THE GABA-BENZODIAZEPINE RECEPTOR COMPLEX IN THE INBRED MOUSE BRAIN

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Previous investigations of the central mechanisms of formation of inherited differences in responses to emotional stress and to benzodiazepine tranquilizers in experiments on C57BL/6 (B6) and BALB/c (C) mice have led to the discovery and description of the phenotypic variants of regulation of the benzodiazepine receptor complex by $Cl^-$ ions [1].

In the present investigation dependence of reception in the $Cl^-$-ionophore site on the GABA receptor was studied on models used previously.

## EXPERIMENTAL METHOD

Experiments were carried out on male C57BL/6 and BALB/c mice weighing 18-20 g and obtained from the "Stolbovaya" nursery. The animals were kept in the laboratory animal house for at least 2 weeks before the experiment began, on a standard diet, and with 12 mice per cage, under conditions of 12 h of darkness and 12 h of daylight. The method of isolating and purifying the brain membrane fraction was described previously [1]. Only freshly isolated preparations were used. The residue was resuspended in 50 mM Tris-citrate buffer, pH 7.4, so that on addition of an aliquot of 1 ml the protein content was 400-500 $\mu$g and the concentration of the radioligand $^{35}$S-*tert*-butylbicyclophosphorothionate ($^{35}$S-TBPT) (from "New England Nuclear," USA, specific radioactivity 87.7 Ci/mmole) was 2 nM. Nonspecific binding, determined in the presence of picrotoxin (final concentration 100 $\mu$M) was about 10% of the total. NaCl in a concentration of 50 or 200 mM was added to the incubation medium. GABA was dissolved in the original Tris-citrate buffer. Aliquots were taken from the mother solution of bicuculline, made up in methanol, so that the final alcohol concentration in the sample did not exceed 1%. Incubation was carried out at 21°C for 90 min. The reaction was stopped by rapid filtration through GF/B filters ("Whatman," England), followed by washing twice with buffer. The conditions of treatment of the filters and counting radioactivity were described previously [1]. Protein was determined by Lowry's method. The results were subjected to statistical analysis by Student's method.

## EXPERIMENTAL RESULTS

Analysis of the curves showing the change in level of $^{35}$S-TBPT bound with brain cell membranes, on the addition of GABA to medium containing different concentrations of NaCl, revealed a decrease in reception of the radioligand which was dependent on the GABA concentration (Fig. 1a, b). Binding of $^{35}$S-TBPT in the presence of $10^{-4}$ M GABA did not differ from the nonspecific level.

The data in Table 1 show that $IC_{50}$ on the addition of GABA was independent of the ionic strength of the incubation medium. The results obtained on brain membrane preparations from animals of the two lines were similar. The character of the effect of GABA on binding of $^{35}$S-TBPT and the values of $IC_{50}$ determined in this investigation agreed with previous results [6],

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TABLE 1. Interlinear Differences in Values of $IC_{50}$ and $n_x$ Obtained from Curves of Displacement of $^{35}$S-TBPT by GABA in the Presence of Different Concentrations of NaCl

| Parameter | Concentration of NaCl, mM | BALB/c | C57BL/6 |
|---|---|---|---|
| $IC_{50}$ (M) | 50 | $(2,77\pm0,23)\cdot10^{-6}$ ($n$=5) | $(2,11\pm0,21)\cdot10^{-6}$ ($n$=5) |
| | 200 | $(2,82\pm0,20)\cdot10^{-6}$ ($n$=6) | $(2,24\pm0,20)\cdot10^{-6}$ ($n$=6) |
| $n_x$ | 50 | 1,16±0,04 ($n$=5) | 0,99±0,038 ($n$=5) |
| | 200 | 1,23±0,045 ($n$=6) | 1,1±0,05 ($n$=5) |

**Legend.** $n$) Number of animals; horizontal arrow indicates statistically significant differences ($p < 0.05$); vertical arrow denotes statistically significant differences by Student's test for tied pairs ($p < 0.05$).

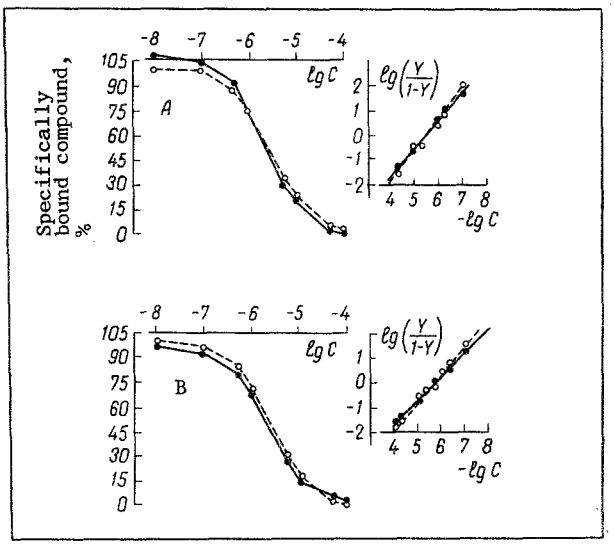


Fig. 1. Effect of GABA on binding of $^{35}$S-TBPT in brain of inbred mice. a) BALB/c; b) C57B1/6; 1) 50 mM NaCl; 2) 200 mM NaCl; I) bound radioligand (in %); C) GABA concentration (in M).

and it can accordingly be concluded that there are no genetic differences with respect to this parameter in the animals of the two lines tested.

Interlinear differences were observed on analysis of the slope of the curves showing the fall in the level of bound $^{35}$S-TBPT, and described by Hill's pseudocoefficient ($n_x$). In medium containing 50 mM NaCl, the value of $n_x$ was found to be significantly higher in C than in B6 mice. With an increase in ionic strength, $n_x$ of the C animals was unchanged but $n_x$ of the B6 mice increased, in agreement with genetic differences in the regulation of the benzodiazepine receptor complex by $Cl^-$ ions discovered previously [1].

The results given in Fig. 2a, b show that the inhibitory effect of GABA on $^{35}$S-TBPT reception is mediated through the GABA-A-receptor, for addition of bicuculline prevented the effect of the neurotransmitter. Lowering the GABA concentration in the medium regularly shifted the curve of dependence of the bound $^{35}$S-TBPT level on bicuculline concentration to the right, into the region of lower acting concentrations of the antagonist. Changes in reception of the radioligand on the addition of submaximal concentrations of bicuculline ($5\cdot10^{-6}$ and $5\cdot10^{-5}$ M) aroused particular interest. In the present experiments, on the addition of GABA in a concentration of $10^{-5}$ M, the typical picture of displacement of $^{35}$S-TBPT was observed, reflecting antagonism of GABA and bicuculline (Fig. 2a, b). However, on reducing the amount of added GABA to $10^{-6}$ M an unexpected

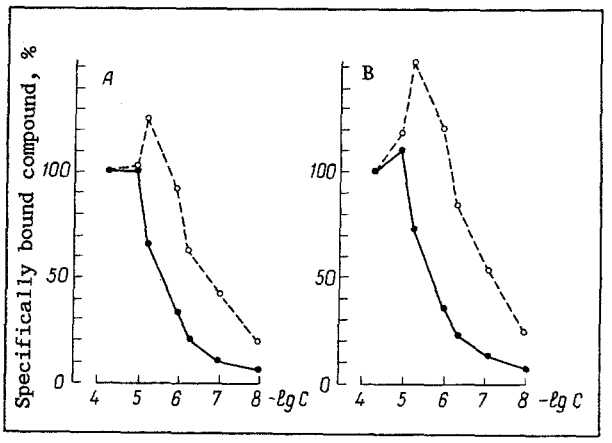


Fig 2. Effect of bicuculline on binding of $^{35}$S-TBPT in brain of inbred mice in the presence of different GABA concentrations. A) BALB/c; B) C57BL/6; 1) GABA ($10^{-5}$ M); 2) GABA ($10^{-6}$ M); C) bicuculline concentration (in M); binding of radioligand in presence of $5 \cdot 10^{-5}$ M bicuculline taken as 100%.

result was obtained: submaximal concentrations of bicuculline stimulated binding of $^{35}$S-TBPT above the control values. The stimulating component was more marked in B6 mice.

No analogous data could be found in the accessible literature, although a biphasic effect (with a stimulating component) of GABA on reception of $^{35}$S-TBPT also was observed in [8].

Despite the difficulty in interpretation of the data, a number of hypotheses can be put forward. First, the characteristics of interaction of the GABA receptor and binding site of the $Cl^-$-ionophore are evidence of the aggregated character of the dependence, which may evidently include both inhibitory and stimulating effects. Second, functional heterogeneity of linking of the GABA-receptor with the $Cl^-$-ionophore must be considered. Finally, on the basis of the generally accepted view that binding of GABA with the receptor opens the $Cl^-$-ionophore and lowers the level of anxiety [5] (on the addition of GABA reception of $^{35}$S-TBPT is depressed), the possibility cannot be ruled out that the increase in binding of $^{35}$S-TBPT, dependent on the GABA receptor, established in this investigation may reflect the opposite process, namely closure of the $Cl^-$-ionophore and a consequent increase of anxiety.

Some support for this hypothesis is given by the interlinear differences observed in this investigation, in the form of the wider range of bicuculline concentrations inducing stronger stimulation of binding of $^{35}$S-TBPT in B6 than in C mice, and this in turn may be evidence of the higher regulatory potential of the benzodiazepine receptor complex in B6 mice. Incidentally, the development of the response to stress and to benzodiazepines in B6 mice also takes place with a higher degree of inertia than in C mice [4]. Attention also must be paid to data [7] indicating a lower GABA concentration in the brain of B6 than of C mice, and in light of the results of the present investigation, this may account for the higher level of ACTH in intact B6 mice than in C mice [2], as an indicator of the initially higher level of anxiety. In turn, ACTH can lead to dissociation of the homogeneous population of GABA-receptors [3].

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