ROLE OF THE Cl^--IONOPHORE REGION IN GABA-BENZODIAZPINE RECEPTOR FUNCTION

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Previously during analysis of hereditary features of the binding capacity of the benzodiazepine receptor, undertaken on inbred C57BL/6 (B6) and BALB/c (C) mice with opposite types of behavior in the "open field" test, it was shown [1] that brain cell membranes of these animals have unequal capacity for ^3H-diazepam reception, depending on the NaCl concentration in the medium.

In the investigation described below binding of ^{35}S-tert-butylbicyclophosphorothionate (^{35}S-TBPT), a specific ligand for the Cl^--ionophore, was studied on models used in the previous experiments.

EXPERIMENTAL METHOD

Experiments were carried out on male B6 and C mice weighing 18-20 g, obtained from the "Stolbovaya" Nursery. The animals were kept under laboratory animal house conditions for at least 2 weeks before the beginning of the experiment, on a standard diet, with 10 animals in a cage and with alternation of 12 h of daylight and 12 h of darkness.

The membrane fraction of the brain was isolated and washed as described previously [1]. Only freshly isolated preparations were used. The residue was resuspended in 50 mM Tris-citrate buffer, pH 7.4, on the basis of the calculation that on the addition of an aliquot with a volume of 1 ml the protein concentration would be 400-600 µg, and the concentration of the ligand ^{35}S-TBPT (from "New England Nuclear," USA, specific radioactivity 87.7 Ci/mmole) would be 2 nM. Nonspecific binding, determined in the presence of picrotoxin (final concentration 100 µM) was about 10% of total binding. In the study of binding of ^3H-diazepam ("Amersham," England, specific radioactivity 94 Ci/mmole) aliquots of suspension contain-

TABLE 1. Analysis of Interlinear Differences in Values of IC_{50} and n_{Hill} Obtained from Curves of Displacement of ^{35}S-TBPT by Picrotoxin, in Different NaCl Concentrations (M ± m)

Parameter	C57BL/6	F_1 (C57BL/6×BALB/c)	BALB/c
IC_{50} (M) 50 mM NaCl	$(3.43\pm0.24)\times10^{-7}$	$(2.51\pm0.20)\times10^{-7}$	$(4.96\pm0.53)\times10^{-7}$
n	5	4	5
200 mM NaCl	$(2.3\pm0.23)\times10^{-7}$ ↕	$(1.69\pm0.04)\times10^{-7}$ ↕	$(2.9\pm0.47)\times10^{-7}$ ↕
n	6	5	5
n_{Hill} 50 mM NaCl	0,86±0,03 ↕	0,74±0,03 ↕	0,9±0,04
200 mM NaCl	1,03±0,04	0,9±0,03	0,88±0,02

Legend. n) Number of animals. Arrows indicate statistically significant differences ($p < 0.05$).

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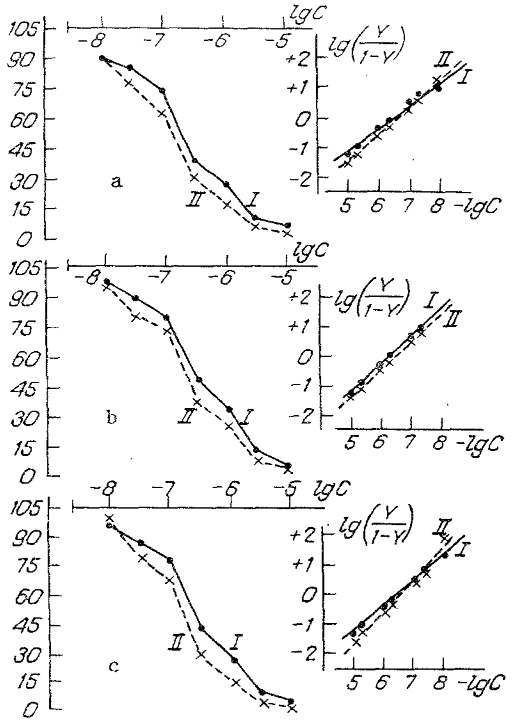

Fig. 1

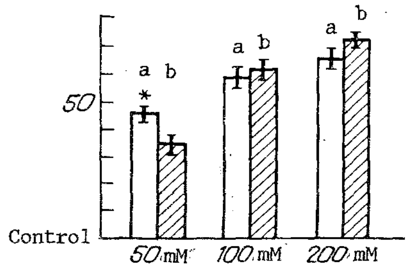

Fig. 2

Fig. 1. Effect of picrotoxin on binding of ^{35}S-TBPT in brain of inbred mice. a) (C57BL/6 × BALB/c)F_1; b) BALB/c; c) C57BL/6. I) 50 mM NaCl; II) 200 mM NaCl. Y) Bound radioligand (in %); C) concentration of picrotoxin (in M). Ordinate, on left: specifically bound compound (in %).

Fig. 2 Effect of NaCl on binding of ^{3}H-diazepam in brain of inbred mice ($M \pm m_X$). a) BALB/c; b) C56BL/6; *) Statistically significant interlinear differences ($p < 0.05$). Abscissa, NaCl; ordinate, stimulation (in %).

ing 200-300 μg protein in a sample with a volume of 1 ml were used; the final concentration of the radioligand was 1 nM. Nonspecific binding was determined by adding an excess of unlabeled diazepam in a concentration of 10 μM. Depending on the series of the experiment, NaCl was added to the incubation medium in final concentrations of 50 or 200 mM. Incubation was carried out at 21°C for 90 min (for ^{35}S-TBPT) and at 0-4°C for 30 min (for ^{3}H-diazepam). The reaction was stopped by rapid filtration through CF/B filters ("Whatman," England), followed by washing twice with cold buffer. The conditions of treatment of the filters and of counting radioactivity were described previously [1]. Protein was determined by Lowry's method. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Curves reflecting displacement of the bound ^{35}S-TBPT by picrotoxin are illustrated in Fig. 1. An increase in the NaCl concentration in the incubation medium led to an increase in affinity of the radioligand for the receptor in both parental lines and also in F_1(B6/C) hybrids. Under these circumstances the value of IC_{50} at minimal NaCl concentration was significantly higher in C mice than in B6 mice, whereas in the F_1-hybrids it was lower than in the B6 parents (Table 1). With an increase in the NaCl concentration to 200 mM, no significant interlinear differences of IC_{50} were observed. It can thus be concluded that with an increase in the NaCl concentration affinity of C mice for the ^{35}S-TBPT receptor rises more sharply than in B6 mice. With respect to the character of these changes the F_1-hybrids corresponded more closely to the B6 parental line. Similar differences between B6 and C mice were found during analysis of dependence of ^{3}H-diazepam reception on the Cl^- ion concentration (Fig. 2). In this case also, strengthening of binding in C mice took place more intensively.

Values of Hill's pseudocoefficient (n_{Hill}) also are given in Table 1. Analysis of values of n_{Hill} obtained in incubation medium with different NaCl concentrations shows that it was significantly increased in the B6 and F_1 animals, and approached the value of 1, whereas in C mice this parameter was unchanged, suggesting the possibility of structural changes in receptors of B6 and F_1-hybrid mice, leading to a decrease in the heterogeneity of the receptor population or of the cooperativeness of interaction of radioligand with receptor, and the absence of such changes in the C animals. Values obtained for IC_{50} of picrotoxin agree with data in the literature [8, 9]. Meanwhile, results pointing to heterogeneity of the ^{35}S-TBPT receptor or the cooperative character of its interaction agree with investigations revealing the polyphasic character of the saturation curves of this radioligand [7].

It can thus be concluded that the essential feature of the inherited differences discovered in interaction of ^{35}S- TBPT with the brain membranes of the inbred animals used as models is the milder character of the changes, depending on an increase in ionic strength of the incubation medium in B6 mice compared with C mice. Another possibility is that a change in affinity for the ligand in B6 animals is accompanied by structural changes in receptors which are not found in C mice.

These results as a whole must be compared with data on the behavior of these animals and changes in various neuroendocrine parameters discovered in them during exposure to emotional stress and administration of benzodiazepine tranquilizers [2-5]. In these cases also, responses recorded in the B6 animals were characterized by greater inertia than those in C mice. Considering that the primary stage of formation of the response to emotional stress may be a change in the ratio between ions in the region of the outer membrane of the neuron [6], these data make it possible to undertake the further analysis of cause and effect relations between the inherited type of emotional-stress response and the character of function of the benzodiazepine receptor complex.

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