IONIC REGULATION OF SPECIFIC ^{3}H-DIAZEPAM BINDING DEPENDING ON EMOTIONAL STRESS REACTION PHENOTYPE

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Previous investigations conducted on inbred C57BL/6 (B6) and Balb/C (BC) mice, differing in their reactions to emotional stress in the open field (OF) test showed that these animals are characterized by a whole range or neurochemical differences, which are clearly manifested during development of the stress response [3-5]. The most important fact is that under OF conditions they may react in different ways to benzodiazepine tranquilizers [7]. In an attempt to elucidate the primary mechanism of formation of differences in the reaction to emotional stress and effects of benzodiazepine, we studied the characteristics of endogenous regulation of specific binding of ^{3}H-diazepam and found that the models used differ with respect to dependence of the stimulation action of NaCl on receptor activity of the benzodiazepine site of the GABA-benzodiazepine receptor complex on concentration [1, 2].

The aim of this investigation was to study ionic regulation of ^{3}H-diazepam reception in the brain of B6 and BC mice against the background of emotional stress in the OF test, and of preliminary injection of benzodiazepine tranquilizers.

EXPERIMENTAL METHOD

Experiments were carried out on male B6 and BC mice weighing 18-20 g. The animals were kept under animal house conditions for at least 2 weeks before the experiment began, on a standard diet, with 10 mice to a cage, and with 12 h of daylight and 12 h of darkness. Isolation of the membrane fraction of the brain and conduct of the radioligand binding followed techniques described previously [1, 2]. As the ligand we used ^{3}H-diazepam (specific activity 71 Ci/mmole, from "Amersham," UK). A model of emotional stress was introduced in the OF test [7]. Benzodiazepine tranquilizers – diazepam and hydazepam (from the A. V. Bogatskii Pharmaceutical Chemical Institute, Academy of Sciences of Ukraine) were injected intraperitoneally as an aqueous suspension with Tween-80, 30 min before the experiment began. The results were subjected to statistical analysis by Student's test for untied and tied pairs.

EXPERIMENTAL RESULTS

In the 1st stage of the series of experiments the ability of different concentrations of NaCl to increase binding of ^{3}H-diazepam with brain membranes of intact and stressed mice was compared in the B6 and BC lines. The curve in Fig. 1a shows that the level of benzodiazepine reception depended strongly on the NaCl concentration in the incubation medium. The curve can be divided into three segments. Within the 1st concentration range (up to 60 mM) the level of binding of the radioligand was increased to 161.8% in BC and up to 154.5% in B6 mice. The stimulating capacity of NaCl within this range was more marked in BC than in B6 mice. In the 2nd segment (from 60 to 100 mM) the curve flattened out on a plateau, and it was only starting from 120 mM that the stimulating effect

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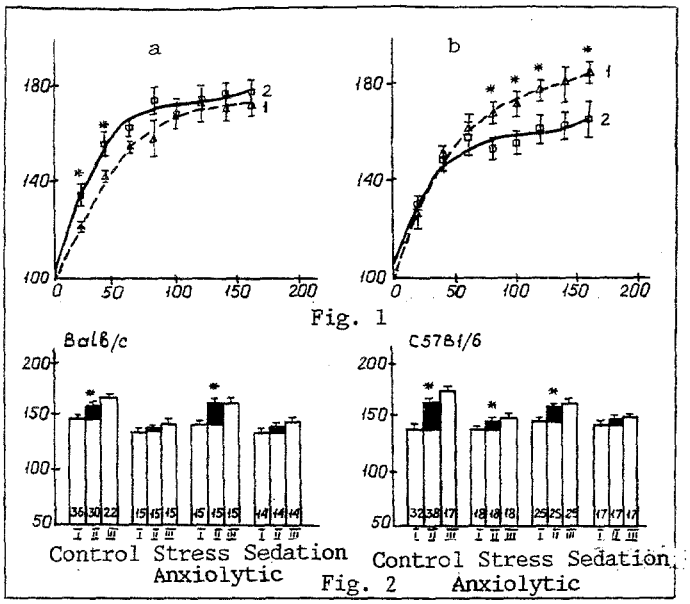

Fig. 1

Fig. 2

Fig. 1. Effect of NaCl on binding of ^{3}H-diazepam with brain membranes of inbred mice (dependence on exposure to emotional stress). Abscissa, NaCl concentration (in mM); ordinate, specific binding (in %). Control level taken as 100%: a) intact animals, b) after stress, in open field test. 1) C57BL/6, 2) Balb/C. Asterisk indicates statistically significant interlinear differences between identical NaCl concentrations ($p < 0.05$). Each point is mean of 5-6 independent experiments.

Fig. 2. Effect of hydazepam and diazepam on ability of NaCl to stimulate binding of ^{3}H-diazepam with brain membranes of control and stressed Balb/C and C57BL/6 mice. Control – intact animals. Stress – control injection of solvent followed by exposure of 3 min in open field. Anxiolytic – preliminary injection of diazepam (0.75 mg/kg) or hydazepam (1 mg/kg). Sedation – preliminary injection of diazepam or hydazepam in a dose of 10 mg/kg I, II, and III) NaCl concentrations (50, 100, and 150 mM respectively). Number of experimental points indicated inside columns. Asterisk indicates statistically significant differences between level of binding in the presence of 100 and 50 mM NaCl.

increased again, to reach 177.6% to for the BC mice and 171.7% to for the B6 mice, with an NaCl concentration of 160 mM.

Results demonstrating the ability of NaCl to enhance benzodiazepine reception in brain membranes obtained from stressed animals are shown in Fig. 1b. In this case, the maximum of stimulation was reached for BC mice at 60 mM NaCl without any significant changes on a further increase in its concentration, and no interlinear differences characteristic of membranes from intact mice were discovered. Conversely, an increase of ^{3}H-diazepam binding, on the addition of NaCl, starting with a concentration of 80 mM and higher, after exposure to emotional stress, was much more marked in the B6 mice. It can accordingly be concluded that not only do interlinear differences in stimulation of benzodiazepine reception by brain membranes obtained from intact animals by NaCl exist, but there are also differences in the character of the change in this process after exposure to emotional stress in the open field (OF) test, that depend on the animals' genotype.

In the next stage of the investigation the changes observed after emotional stress in the ionic regulation of benzodiazepine reception were assessed after preliminary injection of two benzodiazepine tranquilizers, namely diazepam and hydazepam. It was shown previously that in low doses benzodiazepine tranquilizers activate the behavior of B6 mice in the OF test, which was interpreted as an anxiolytic effect. Conversely, larger doses of benzodiazepines had a similar sedative action of mice of both lines [6, 7]. Effects of diazepam and hydazepam in low and

high doses were qualitatively similar for each strain of animals, and they are therefore pooled in Fig. 2. Control injection of the solvent 30 min before the experiment in OF reduced the absolute values of the stimulating action of NaCl on benzodiazepine reception in the two lines of mice. However, in the BC line, dependence of this effect on concentration, characteristic of intact animals, disappeared under these circumstances. Conversely, in B6 mice this dependence was preserved. The anxiolytic action, developing in BC mice after injection of benzodiazepines in low doses, was accompanied by restoration of the ability of NaCl to increase the level of binding of ^{3}H-diazepam, especially in concentrations of 100 and 150 mM, compared with the injection control. Dependence of the effects of NaCl on concentration, characteristic of brain membranes of intact BC mice, was again recorded in this cage, Injection of diazepam and hydazepam in low doses into B6 mice had no appreciable effect on dependence of the action of the salt on concentration, although it increased the absolute values of its stimulating capacity.

When tranquilizers were used in doses giving rise to a sedative action in BC and B6 mice the level of stimulation of benzodiazepine reception characteristic of each NaCl concentration was lowered, and the concentration dependence of the effect discovered previously disappeared in both lines of mice. It can accordingly be concluded that the ability of NaCl to increase binding of ^{3}H-diazepam with brain membranes is a physiologically important parameter which reflects hereditary differences in responses to emotional stress and has its own specific pattern for the predominantly anxiolytic or predominantly sedative action of benzodiazepine tranquilizers.

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