Benzodiazepine Reception in C57Bl/6 and BALB/c Mice Depending on the Type of Stress Factor

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Specific binding of ^{3}H-flunitrazepam with synaptosomal membranes after exposure to open field test and "contact with predator" test was measured in C57Bl/6 and BALB/c mice. Stress-induced decrease in benzodiazepine binding after open field test was observed only in BALB/c mice and after contact with predator in both animal strains.

Key Words: *$GABA_A$-receptor; ^{3}H- flunitrazepam; inbred mice; emotional stress; open field; contact with predator*

Emotional stress modeled in a number of experiments decreased binding of labeled ligands by $GABA_A$-receptor benzodiazepine site [6,7]. Neuroimaging methods revealed similar effects in patients with panic attacks [5] and posttraumatic stress disorders [9]. Our previous studies showed that exposure to emotional stress in the open field (OF) test decreased receptor binding of ^{3}H-diazepam in Balb/c mice, but not in C57Bl/6 mice [2]. BALB/c mice are characterized by freezing reaction in OF test, which is interpreted as a fear reaction, whereas C57Bl/6 mice demonstrate increased motor activity and exploratory activity in OF. On the basis of these interstrain differences C57Bl/6 and BALB/c mice were defined by some authors as stress-resistant and stress-liable strains, respectively [1]. It remains unclear, whether stress-induced decrease in benzodiazepine receptor binding is a specific feature of Balb/c mice, or this effect does not depend on animal genotype and is associated with fear reaction, if the latter exceeds a threshold value. Here we studied specific binding of ^{3}H-flunitrazepam by membrane fraction from the brain of C57Bl/6 and BALB/c mice after OF test and "contact with predator" test (CPT).

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MATERIALS AND METHODS

Experiments were carried out on male C57Bl/6 and BALB/c mice weighting 18-20 g, obtained from Pushino nursery (affiliate of M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences).

The animals were kept in a vivarium of Laboratory of Pharmacogenetics, V. V. Zakusov Institute of Pharmacology, 20 animals per 3H cage for 10 days before the experiment with water and food *ad libitum* and non-inverted 12-hour light regimen.

Mongrel 2-year-old female cat was used in CPT experiments.

Exposure to emotional stress was performed in OF using light flash in modification [1].

In CPT, control mouse was placed one by one into a transparent Plexiglas cage 40×40×20 cm with transparent cover for 5 min and then decapitated.

Experimental mouse was placed into the cage and then the cat was allowed to approach the cage (the cat demonstrated specific features of predator behavior). After 5-min contact, the mouse was decapitated.

After decapitation, the brain was immediately removed and stem structures and the cerebellum were isolated. Remained part of the brain was homogenized in 20 ml cold Tris-HCl buffer solution (4°C, 50 mM pH 7.4) and centrifuged at 54,000*g* for 25 min using Beckman centrifuge. The supernatant was discarded.

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The pellet was resuspended by homogenization in the initial volume of the buffer and centrifuged under the same conditions. This procedure was repeated 3 times. For radioligand assay, the pellet was resuspended in 20 ml cold buffer solution and incubated on ice for 30 min in 3 repeats for each brain sample. The incubation medium contained 250 µl membrane fraction, 50 µl 10 nM [N-methil-^{3}H]-flunitrazepam (87.0 Ci/mmol; Amersham Bioscience). Non-specific binding was measured by adding 50 µl 400 µM diazepam (Sigma) in 5% ethanol to samples. The volume was brought to 500 µl with cold buffer solution (200 and 150 µl). Benzodiazepine binding reaction was stopped by vacuum filtration on fiberglass filters (GF/B Whatman). Filter radioactivity was measured on a Beckman scintillation counter after adding in 3 ml scintillation fluid. Protein content in the final suspension was assessed by Lowry method [8], using albumin (Bovine, albumin; SIGMA Chemical Co.) as the standard. The results were presented in DPM/µg of protein.

Statistical processing of results obtained was carried out using Mann—Whitney *U* test for independent samples.

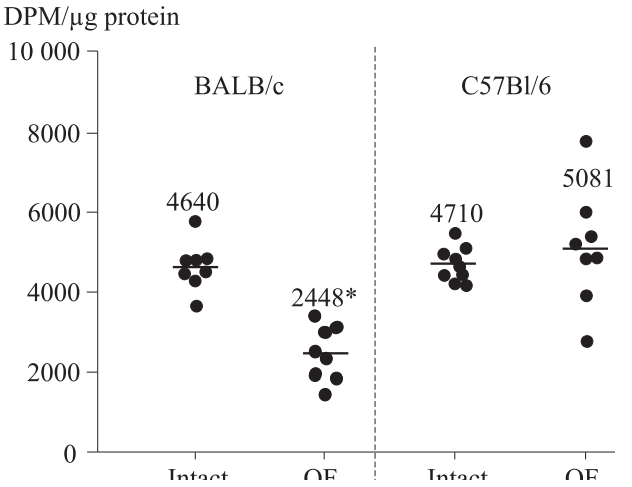

Fig. 1. Specific binding of ^{3}H-flunitrazepam by membrane fraction (P_1+P_2) of the brain from BALB/c and C57Bl/6 mice before and after exposure to emotional stress in OF. **p*<0.05 compared to intact mice.

RESULTS

Analysis of OF behavioral of C57Bl/6 and BALB/c mice confirmed previously revealed interstrain differences in their emotional stress reaction [1] by the criterion of motor activity and defecation (Table 1).

Visual monitoring of mouse behavior in CPT showed that control animals (not contacting with the predator) freely moved and demonstrated specific signs of exploratory activity, which were more pronounced in C57Bl/6 mice.

Ethologically marked signs of anxiety and fear were observed in experimental BALB/c mice. In the presence of the predator, their motor activity decreased, long freezing episodes appeared, the animals often lied flat and dug into the cuttings. In C57Bl/6 mice, exploratory activity significantly decreased, short freezing episodes alternated with active defensive behavior, runs to the opposite from the predator side of the cage. These results correspond to those in studies performed using similar methods [4].

Specific binding of [N-methyl-^{3}H]-flunitrazepam to brain membranes from BALB/c mice significantly decreased after exposure to OF, while in C57Bl/6 mice parameters of receptor binding remained unchanged (Fig. 1). Similar results were obtained previously in experiments with labeled ligand ^{3}H-diazepam [3].

Binding of [N-methyl-^{3}H]-flunitrazepam by brain membranes from C57Bl/6 and BALB/c mice exposed to emotional stress in CPT is shown on Figure 2. Control parameters of ligand binding in both mouse strains were similar and did not differ from values obtained in experimental series I, which attests to good reproducibility of the results. Contact with the predator decreased in benzodiazepine reception in both BALB/c and C57Bl/6 mice, but this decrease was more pronounced in BALB/c mice (Fig. 2). Published data suggest that contact with the predator induces panic reaction in various laboratory mouse strains [10], which corresponds to our findings. The decrease in

TABLE 1. Behavior of Balb/c and C57Bl/6 Mice in OF (P. M. Borodin Modification, *M*±*SE*)

Strain	Types of motor activity					Number of defecations
	peripheral activity	central activity	center	vertical activity	total motor activity	
Balb/c (*n*=10)	13.4±1.7	0.0±0.0	0.0±0.0	0.0±0.0	13.4±1.7	0.6±0.2
C57Bl/6 (*n*=10)	66.5±5.4*	24.4±2.3*	2.0±0.5*	14.6±1.6*	107.5±7.9*	1.5±0.5

Note. **p*<0.05 compared to Balb/c mice.

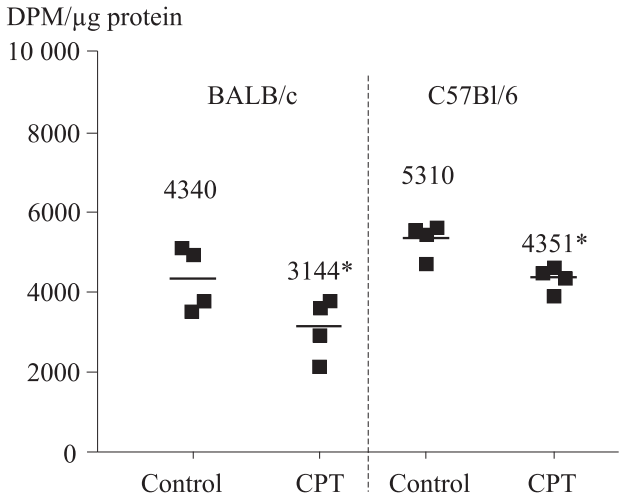

Fig. 2. Specific binding of ^{3}H-flunitrazepam by membrane fraction (P_1+P_2) of the brain from BALB/c and C57Bl/6 mice before and after exposure to emotional stress in CPT. *p<0.05 compared to the control.

[N-methyl-^{3}H]-flunitrazepam binding in C57Bl/6 and BALB/c mice after CPT and only in BALB/c mice after OF testing suggests that the stress-induced decrease in binding properties of $GABA_A$-receptor benzodiazepine site is not genotype-specific, but depends on strength of the stress factor and can be used as a neurochemical marker of fear reaction appearance.

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