GENETICS

Intracerebral Administration of Brain-Derived Neurotrophic Factor (BDNF) Reduces Severity of Cataleptic Freezing in Mice with Genetic Predisposition to Catalepsy

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Single administration of brain-derived neurotrophic factor (BDNF) into the lateral ventricle of ASC mice (Antidepressant Sensitive Catalepsy), a model of depression-like state, significantly decreased predisposition to cataleptic freezing in these animals. These findings indicate that BDNF can appear as a promising antidepressant of new generation and that ASC mice can be used as an adequate model for investigations of the mechanisms of behavior modification by BDNF.

Key Words: *brain-derived neurotrophic factor (BDNF); catalepsy; forced swimming test; mouse*

According to WHO data, depressive psychosis is one of four the most severe human diseases; 12.7% males and 21.3% females in developed countries suffer from depressive psychosis. Significant efforts are now directed towards evaluation of the role of BDNF in the pathogenesis of depressive disorders; depression was hypothesized to be associated with neurodegenerative processes in the hippocampus and cortex [6]. BDNF is of special interest, because its serum level is a sensitive marker of antidepressant efficacy in depressive patients [11]. On the other hand, chronic treatment with antidepressant and electroconvulsive therapy providing antidepressant effects increase BDNF expression in the hippocampus and cortex [12,13]. Thus, the possibility of using BDNF as an antidepressant is a promising direction for further investigations.

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It was previously found, that selection for high predisposition to catalepsy and freezing reaction, *i.e.* long-term immobility and inability to correct externally formed unnatural posture, results in the development of the depression-like state in mice [1]. In addition, ASC mice (Antidepressant Sensitive Catalepsy) obtained as the result of selection exhibit high hereditary predisposition to catalepsy, which can be controlled by chronic, but not acute administration of classical tricyclic antidepressant imipramine [3] and selective serotonin reuptake inhibitors fluoxetine currently used in clinical practice [2]. This agrees with the data of clinical observation on delayed therapeutic effect of antidepressants [4]. Mouse model of genetic predisposition to catalepsy satisfies two major requirements for experimental models of depression: face validity (similarity of symptoms of depressive disorder with depression-like features of the model) and predictive validity (similarity in the responses to antidepressant administration), and appear to be a convenient tool for

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investigation of depression development mechanisms and antidepressant action [2].

The aim of this study was investigation of the effects of central BDNF administration on the severity of catalepsy and behavior in forced swimming test and open field test in ASC mice.

MATERIALS AND METHODS

Experiments were carried out on 29 adult male ASC mice. The ASC line was recently produced in the Laboratory of Neurogenomics of Behavior, Institute of Cytology and Genetics, by selection for high predisposition to catalepsy. First, F_1 hybrids were obtained from cataleptic CBA/Lac mice and non-cataleptic AKR/J and then these hybrids were mated with parental CBA/Lac strain. Backcrosses served as the parent material for further selection. Cataleptic mice were selected among them and first generation of selection was obtained. Starting from the 5th generation, the selection was combined with sibling inbreeding, from the 8th generation the selection was stopped, but inbreeding was continued [9]. Animals of the 30th generation aging 3-4 months (body weight 28±1 g) were used in the study. The animals were kept in 50×30×25 cm cages (5-6 animals per cage) under natural illumination and 22±2°C with *ad libitum* food and water supply. One day before the experiment the animals were placed in individual 50×30×25 cm cages to eliminate group effects. The mice were divided into 2 weight and age-matched groups: control (n=14) and experimental (BDNF; n=15). All manipulations with animals were performed in accordance with international regulation for handling animals (European Community Directive 86/309, December 24, 1986).

BDNF (Sigma) was dissolved in physiological saline and injected (0.3 μg BDNF in 5 μl saline) into the left cerebral ventricle (AP: -0.5 mm, L: -1.6 mm, DV: 2 mm). Control animals were injected with physiological saline in the same volume. Since antidepressant action of BDNF is presumably associated with its effects on neurogenesis and neurodegenaration [6], the effects of BDNF administration were investigated 3-7 days after injection.

Forced swimming test was performed 3 days after administration of BDNF or physiological saline, using an apparatus consisting of rectangle plastic reservoir with lateral matted nontransparent sides and transparent bottom (14×14×22 cm), inverted illumination system and computer registration system EthoStudio. The animal was placed into the reservoir $^3/_4$ filled with water (25-26°C). After 120-sec adaptation, the total immobility time was recorded over 4 min [5].

Open field test was performed 5 days after administration of BDNF or physiological saline, using an apparatus consisting of a round arena 40 cm in diameter, inverted illumination system, and computer registration system EthoStudio [10]. The animal was placed on the arena near the wall and path length (cm), probability of visits to the central zone, number of rearings, number and duration of grooming episodes, and number of defecations were recorded over 5 min.

Catalepsy test [8] was performed 1 week after administration of BDNF or physiological saline. Catalepsy was induced by pinching the skin at the nape of the neck, after which the animal was placed on two parallel bars located 5 cm from each other at an angle of 45°. The test was positive, if the mouse retained unnatural imposed posture for at least 20 sec. The test duration did not exceed 120 sec, after which the animal was returned to its cage. A total of 10 tests were performed with intervals of 1-2 min. The animal demonstrating 3 positive tests were regarded as cataleptic. For calculations of the mean freezing time, three tests with maximum immobility time were used. The percentage of cataleptics in the group was also calculated.

The results were presented as $M\pm SEM$ and compared using one-way ANOVA. The percent of cataleptic animals in groups was compared using exact two-way Fisher test.

RESULTS

Central administration of BDNF significantly reduced the percentage of cataleptic animals (p=0.007; Fig. 1, *a*). Only 21% animals in the experimental group exhibit catalepsy, whereas the percent of cataleptic animals in the control group was 77%, which is close to that in intact group of ASC mice. BDNF administration also slightly shortened freezing time ($F_{1,25}$=3.92, p=0.059; Fig. 1, *b*). Similar to classic antidepressants

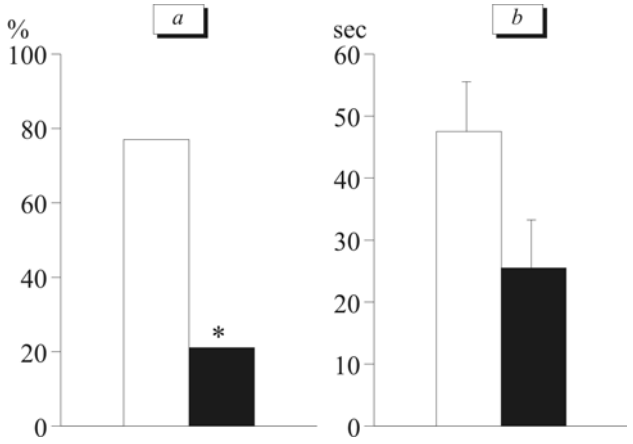

Fig. 1. Effects of central administration of BDNF on catalepsy evidences in ASC mice. *a*) percentage of cataleptic animals, *b*) freezing time. Light bars: mice from control group; dark bars: mice from experimental group. *p<0.01 compared to the control group.

imipramine and fluoxetine [2,3], BDNF suppressed manifestation of catalepsy in ASC mice. However, apart from fluoxetine and imipramine, this effect was attained after single administration of BDNF and was more pronounced (the number of animals with catalepsy decreased by 56% in comparison with 29% and 26% after chronic imipramine and fluoxetine administration, respectively). It should be noted, that excessive catalepsy manifestation in humans is a symptom of some severe mental and neurological diseases, such as depression and schizophrenia, and determined by marked changes in the nervous system. The observed catalepsy-inhibiting effect of BDNF was probably associated with activation of neurogenesis and regeneration processes in the brain induced by the neurotrophic factor and restoring neuromental alterations appeared during selection.

Open field test revealed no significant changes in motor activity and anxiety: BDNF administration did not significantly change the path length (horizontal motor activity) and rearing (vertical motor activity) and probability of entries into the central zone (anxiety parameter; Table 1). Moreover, there were no changes in emotional parameters (number of defecations and number and duration of grooming episodes). This suggests that catalepsy-inhibiting effect of BDNF is specific and not associated with changes in motor activity, anxiety, and emotional state.

BDNF administration did not significantly affect immobility time in the forced swimming test: 114.9±9.6 sec in the experimental group vs. 121.6±11.6 sec in the control ($F_{1,25}$<1). This agrees with previous data demonstrating inefficiency of chronic administration of imipramine on behavior of ASC mice in this test [3]. At the same time, experiments on rats showed that BDNF administration reduced immobility time and increased time of active swimming in the forced swimming test [7,14]. It should be noted that in these studies usual "normal" animals were used, and the effect was observed at higher dose (1 μg per animal) [7] than in the present study or after local BDNF administration into the hippocampus [14]. Since it is known that antidepressants have no effect on healthy people [15], behavioral changes in "normal" rats can be associated with other non-antidepressant mechanisms. The model of genetic predisposition to catalepsy, within which BDNF reduces pathological manifestations, seems to be more suitable for evaluation of mechanisms of the effect neurotrophic factor on behavior.

Thus, BDNF reduced the severity of pathological genetically-determined behavior in ASC mice proposed as the model of depression-like state. The results of this study indicate that BDNF may appear as a promising antidepressant of new generation, and ASC mice can be used as convenient and adequate model for investigation of mechanisms of behavior modification by BDNF.

TABLE 1. Behavior of ASC Mice in Open Field Test 5 Days after Central Administration of BDNF

Parameter	Control	BDNF
Path length, cm	865.5±73.9	831.4±67.3
Probability of central position	0.14±0.02	0.13±0.02
Rearings	10.80±1.78	11.60±1.78
Grooming number	2.2±0.2	1.9±0.2
Grooming duration, sec	4.10±0.73	4.40±0.73
Defecation number	3.40±0.49	2.70±0.49

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