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Effect of Enterosorbent Noolith on Behavior and Serotonin (1A) Receptors in Mouse Brain

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Protective properties of a new enterosorbent noolith (lithium ions immobilized on mineral matrix) were studied in C57Bl/6J mice predisposed to depression caused by intermale confrontations. The drug was administered daily for 15 days after the 5th confrontation and then the animals were tested in the forced swimming test. The number of specific ^{3}H-8-OH-DPAT binding sites in 3 brain regions was determined. It is shown that noolith produced an antidepressive effect manifested in decreased immobility time in the Porsolt test. Moreover, noolith reduced the number of 1A-serotonin receptors in the frontal cortex and hypothalamus. It is concluded that noolith possesses protective properties.

Key Words: *noolith; depression; mice; brain structures; serotonin receptors*

Sorption methods of detoxification, such as hemo-, entero-, and application sorption are now widely used in medicine [3]. During contact with biological fluids sorbents apart from adsorption trigger cascade reactions, enhance ion exchange, donor-acceptor interactions, catalytic and biotransformations. Gastrointestinal sorption (enterosorption), a simple and effective method of detoxification with nonspecific sorbents, is used in the treatment of some diseases and poisonings and correction of pathological states associated with endo- and exotoxicoses. Enterosorption is a component of complex prophylaxis, sanitation, and endoecological rehabilitation. Immobilization of various compounds on the surface of sorbents imparts new properties and open new possibilities for their application.

Enterosorbent noolith [1] is a mineral matrix with optimal texture parameters and immobilized lithium-containing compound on its surface. Enterosorbent provides sustained release of potent normothymic lithium into biological fluids and acts as a detoxifying factor preventing complications and side effects associated with lithium overdose.

Preliminary experiments on mice showed beneficial effect of noolith on the behavior of intact animals in various tests [2]. The efficiency of noolith in mice with pronounced experimental depression was also shown [1]. It should be emphasized that noolith shortens the time of immobility [1,2] in the forced swimming test [11]. In light of this it is interesting to evaluate the effect of noolith under conditions of persistent action of a negative emotional factor. In the present study C57Bl/6J mice with experimental depression induced by 20-day intermale confrontations [7,8] were used. Depressive animals exhibit pronounced changes in behavior. For instance, depressive mice show increased immobility time [7,8] in the forced swimming test regarded by some authors as a test for depression [5-7]. A key role in the development of this pathology in played by the serotoninergic system of the brain [4,8]. In this context 5-HT$_{1A}$ receptors are of particu-

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lar interest, because many authors showed antidepressive action of 5-HT_{1A} receptor agonists both in clinical practice [15] and experiments [14]. Tricyclic antidepressants (imipramine, desipramine) and inhibitors of serotonin uptake (citalopram, fluoxetine) change animal behavior acting via 5-HT_{1A} receptors [13].

The aim of the present study was to examine the protective properties of enterosorbent noolith in C57Bl/6J mice.

MATERIALS AND METHODS

Adult 2.5-3-month-old male C57Bl/6J mice weighing 24-26 g were used in the experiments. The animals were kept under standard vivarium conditions (Institute of Cytology and Genetics) at 12-h day-night regimen and free access to water and food (standard granulated forage, Agricultural Technologies, Moscow). Each experimental group consisted of 10-12 animals.

Experimental depression in mice was modeled by the method of sensory contact [7]. The mice were placed in pairs into cages (28×14×10) divided by a perforated wall (sensory contact). After 3 days, the wall was removed, which allowed antagonistic conflicts between males. These confrontations were repeated daily in the afternoon. Social defeat during 20 days led to the formation of a victim group characterized by depressive behavior. For potentiation of the effect of defeat, the victim and its aggressive partner were kept in one cage separated with a transparent perforated wall.

After 5 confrontations, the victims were divided into 2 groups; group I received noolith (0.0133 g in 0.3 ml 2% starch gel) for 15 days, daily, at 10.00 a.m., while group II received placebo (0.3 ml 2% starch gel).

After 15 days (confrontations were continued during treatment), the mice were examined in the forced swimming test routinely used for antidepressant screening [16]. To this end, each mouse was placed in a 1-liter glass vessel filled with water (25±1°C) up to a 9-cm level. The time of complete immobility (motionless hanging in water) was recorded for 5 min.

One day after testing the mice were decapitated. Brain structures (frontal cortex, hippocampus, and hypothalamus) were isolated on ice, immediately frozen in liquid nitrogen, and stored at -70°C. The number of 5-HT_{1A} receptors was determined by the radioligand binding assay [9] with some modifications. Brain specimens were homogenized (1:50-1:80 w/v ratio depending on the structure) in cold 50 mM Tris-HCl buffer (pH 7.6 at 22°C). The homogenate was centrifuged 20 min at 12,500 rpm and 4°C in a Kontron T42K centrifuge. The pellet was homogenized in a fresh portion of buffer, incubated 10 min at 37°C, and re-centrifuged. The resultant pellet was resuspended in the incubation medium containing 50 mM Tris-HCl buffer (pH 7.6 at 22°C) supplemented with 4 mM $CaCl_2$, 10 μM pargyline, and 0.1% ascorbic acid. The obtained suspension was transferred to 2 tubes (0.9 ml each) for total and nonspecific (in the presence of a 10 μM serotonin (Serva) as the competitor) binding of 2 nmol ^{3}H-8-OH-DPAT (221 Ci/mmol, Amersham). All samples were incubated 10 min at 37°C. The reaction was stopped by filtration of the suspensions through fine fiber glass filters GF/B (Whatman) under vacuum. The filters were washed three times with 5 ml cold Tris-HCl (pH 7.6 at 22°C), dried, and transferred into vials with dioxane scintillator. Radioactivity was measured on a Rackbeta 1209 scintillation counter (counting efficacy 42-43%). The number of ^{3}H-8-OH-DPAT specific binding sites (5-HT_{1A} receptors) was expressed in fmol/mg protein. Protein content was determined by the method of Lowry.

The data obtained in behavioral test and biochemical analysis were processed statistically by nonparametric Wilcoxon—Mann—Whitney test using Statistica software.

RESULTS

The forced swimming test revealed a significant effect of noolith manifested in decreased immobility time in mice receiving enterosorbent compared to mice receiving placebo ($p<0.05$, Fig. 1, *a*). Since immobility time is regarded as measure of depression [5,7] and the test is sometimes called despair test [10,16], it is possible to conclude that noolith produces an antidepressive effect and prevents the development of depression. These results agree with previous data on reduced immobility time in the forced swimming test after 14-day noolith administration after 20 confrontations in depressive victims (20 days of intermale confrontations) [1]. Thus, both experiments showed that noolith possesses not only therapeutic, but also protective properties. Antidepressive effect of enterosorbent noolith probably depends on the presence of lithium ions in its structure. Lithium preparations are widely used for the treatment of depressions in combined antidepressant therapy. Antidepressive lithium effect comparable to that of tricyclic antidepressants and monoamine oxidase inhibitors [10] was also shown on various animal models of experimental depression [12].

Systemic noolith administration decreased the number of ^{3}H-8-OH-DPAT specific binding sites in the frontal cortex and hypothalamus compared to mice receiving placebo ($p<0.01$ and $p<0.05$, respectively, Fig. 1, *b*), while in the hippocampus their number was similar in both groups (Fig. 1, *b*). It was demonstrated that lithium preparations and 5-HT_{1A} receptor agonists (8-OH-DPAT, gepirone, buspirone) decreasing

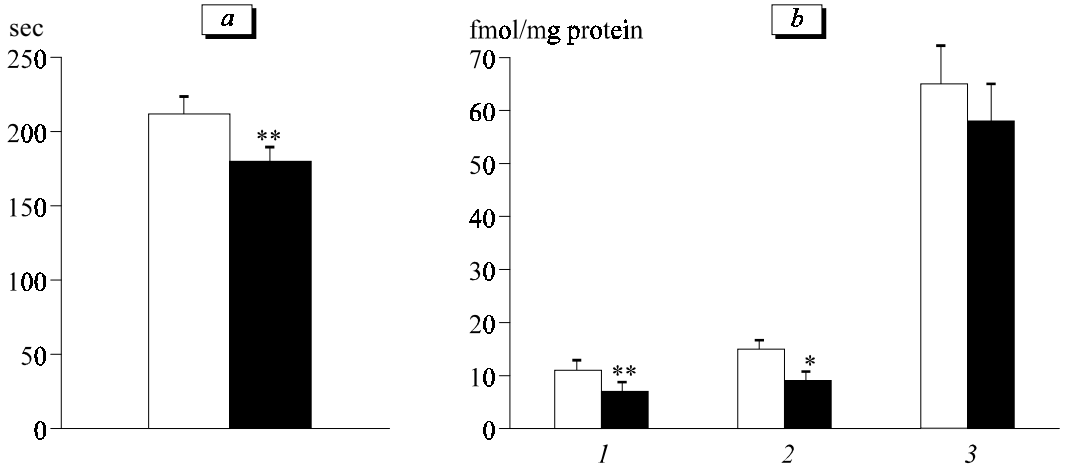

Fig. 1. Effect of enterosorbent noolith on immobility time in the forced swimming test (*a*) and ^{3}H-OH-DPAT binding with 1A-serotonin receptors (*b*) in the frontal cortex (*1*), hypothalamus (*2*), and hippocampus (*3*) in mice. Open bars: placebo; dark bars: noolith. **p*<0.01, ***p*<0.05 compared to placebo group.

immobility time in the forced swimming test act via 5-HT_{1A} and 5-HT_{1B} receptors [10,12,13]. Our experiments revealed a relationship between antidepressive properties of enterosorbent noolith and the number of 5-HT_{1A} receptors in the frontal cortex and hypothalamus.

Thus, enterosorbent noolith showed protective properties. It produced an antidepressive effect decreasing immobility time in the forced swimming test. Noolith reduced the number of 5-HT_{1A} receptors in the frontal cortex and hypothalamus in victims.

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