Effect of Imipramine on the Behavior and Cerebral 5-HT$_{1A}$ Serotonin Receptors in Mice Genetically Predisposed to Catalepsy

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Acute injection of imipramine to NPK mice hereditary predisposed to pinching catalepsy reduced immobility in the forced swimming test, but had no effect on catalepsy. Chronic treatment with imipramine reduced the severity of catalepsy and functional activity of 5-HT$_{1A}$ serotonin receptors, but did not modify their expression in the hippocampus. NPK mice can be a convenient model for studies of the effects of antidepressant.

Key Words: *imipramine; pinching catalepsy; 5-HT$_{1A}$ serotonin receptors; forced swimming test*

Catalepsy (freezing reaction) is a syndrome of many severe nervous and mental human disorders [13]; its manifestation in rats and mice indicates significant changes in the nervous system. GK rat strain with genetic predisposition to catalepsy was derived as a result of many-year selection [1]; these animals are characterized by depression-like behavioral features and changes in the cerebral serotonin system in comparison with Wistar rats [2]. Genetically determined cataleptic reaction in GK rats is sensitive to chronic (but not acute) injection of imipramine (antidepressant) [5], while injection of flezinoxane (nonspecific 5-HT$_{1A}$ serotonin receptor agonist), characterized by a clinical antidepressive effect, appreciably reduced the severity of catalepsy [8], this prompting the use of this animal strain as a model of endogenous depression [2]. In mice catalepsy is induced by a succession of pinching the withers skin (pinching catalepsy) [9, 11]. Recently, an NPK mouse strain was selected at Institute of Cytology and Genetics from a population of back-crosses between the cataleptic CBA and noncataleptic AKR strains. These NPK mice with hereditary predisposition to catalepsy are characterized by extremely high (85%) predisposition to pinching catalepsy and many depression-like behavioral features.

We studied the effects of acute and chronic treatment with imipramine on the severity of catalepsy and behavior in the forced swimming and open field tests and on functional activity and expression of 5-HT$_{1A}$ serotonin receptors in the hippocampus of NPK mice.

MATERIALS AND METHODS

The NPK mouse strain was bred by A. V. Kulikov at Laboratory of Behavioral Neurogenomics, Institute of Cytology and Genetics. CBA-AKR hybrids were crossed with parental CBA strain, and the resultant back-crosses formed the population for further selection of cataleptic mice from different families; the first generation of selection was thus

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obtained. Starting from the 5th generation, selection was combined with brother/sister inbreeding. Experiments were carried out on 2-month-old adult male NPK mice (n=54) of the 10th generation (26.4±0.8 g). During the experiment the mice were kept in isolation with standard illumination and temperature. The animals were divided into control and experimental (imipramine) groups. The effect of acute injection of imipramine (Sigma) on behavior was evaluated during the first 3 days of daily intraperitoneal injections of the drug in a dose of 25 mg/kg. Controls were injected with normal saline in the same volume. The effect of chronic treatment with the drug was evaluated after 10 days. The mice were tested 3 days after the end of imipramine treatment in order to rule out the effect of acute treatment. Behavior in catalepsy test, open field test, and forced swimming (Porsolt) test was evaluated.

Catalepsy was induced by 5-sec pinching the skin of the neck, after which the animal was placed onto two parallel bars positioned at a 45° angle at different height [9,11]. The test was considered positive if the mouse retained this awkward postured for at least 20 sec. The duration of the test was 120 sec, after which the animal was put back into the cage. The tests (n=10) were carried out at 1-2 min intervals. Animals with 3 positive tests of 10 were considered as cataleptics [9].

Porsolt test is the classical test for depressive-like behavior and antidepressant activity [12]. The mouse for this test was placed into a transparent plastic cube (18×18×23 cm) filled with water (25.5°C) by $^2/_3$ of the volume. After 40-min adaptation animal behavior was digitally recorded. The duration of immobility (sec) was measured using EthoStudio software [4].

In order to rule out the possibility of relationship between the observed effects and changes in total motor activity, open field test was carried out in an open white plastic box (80×80×20 cm) illuminated with a 300 W lamp. The animal was placed near the wall of the box in the middle of this wall and behavior was tested for 5 min. The floor of the box was divided into 64 squares. The test was digitally recorded and analyzed using an EthoStudio software [4]. Horizontal (number of crossed squares) and vertical (number of rearings) motor activity was evaluated.

5-HT_{1A} presynaptic autoreceptors of the midbrain serotonin neurons and peripheral 5-HT_{1A} receptors of postsynaptic location are believed to play an important role in the mechanisms of antidepressant action [6]. We studied the effect of chronic treatment with imipramine on functional activity of the midbrain 5-HT_{1A} autoreceptors and on expression of 5-HT_{1A} receptor gene in the hippocampus (the region of the greatest density of postsynaptic 5-HT_{1A} receptors) in NPK mice. Functional activity of 5-HT_{1A} receptors was evaluated by the degree of body temperature decrease after injection of 8-hydroxy-2-(dipropylamino)tetraline (8-OH DPAT; Sigma), agonist of this receptor type. After measuring rectal temperature, the mice (12 from each group) were injected with 8-OH DPAT (1 mg/kg intraperitoneally) and after 30 min the temperature was measured again.

Other animals were decapitated 3 days after the last behavioral test. The brain was removed on the cold, the hippocampus was isolated, placed into liquid nitrogen, and stored at -66°C. Total RNA was isolated and qualitative reverse transcription PCT were carried out as described previously [10]. The level of 5-HT_{1A} receptor mRNA expression in the hippocampus was presented as the number of copies per 100 copies of a house keeping gene (glyceraldehyde-3-phosphate dehydrogenase), which served as the internal standard for reverse transcription. C57BL/6 mouse DNA served as the external reference for evaluating the number of mRNA copies of the appropriate genes per 1 μl cDNA [10].

The percentage of cataleptics in the groups was compared using χ^2 test. Data on duration of immobility in Porsolt test, parameters of motor activity in the open field test, functional activity and expression of 5-HT_{1A} receptors were presented as $M\pm m$ and compared using Student's t test for independent samplings.

RESULTS

A single injection of imipramine did not change the number of cataleptic animals (63% in the control *vs.* 44.4% in the imipramine group), while chronic treatment with the antidepressant significantly reduced predisposition to catalepsy (76.9% in the control and 48% in the imipramine group; p<0.05). Porsolt's test demonstrated acute effect of imipramine: the drug appreciably shortened the duration of immobility (118.2±5.8 sec in the control *vs.* 96.0±6.6 sec after imipramine; p<0.05), while chronic treatment induced no changes. In the open field test the groups did not differ by the number of crossed squares in the acute (83.2±8.8 in control *vs.* 60.7±10.5 after imipramine) and chronic experiment (77.8±8.6 in control and 78.3±9.1 after imipramine). Acute injection of imipramine reduced vertical motor activity (number of rearings) in NPK mice (5.0±0.8 in the control and 2.2±0.5 in the imipramine group; p<0.01), while chronic treatment caused no effect of this kind (4±1 and 3.4±0.6 in control and imipramine groups, respectively). The

results of the open field test indicate that imipramine effects in other tests are not associated with the increase in total motor activity.

The body temperature drop after injection of 8-OH-DPAT, reflecting functional activity of 5-HT_{1A} presynaptic autoreceptors, was significantly less expressed in animals receiving chronic imipramine treatment (2.3±0.3 in the control and 1.4±0.3 in the imipramine groups; $p<0.05$). Study of 5-HT_{1A} receptor mRNA expression in the hippocampus showed no difference between the groups (16.0±1.9 and 17.3±2.9 in control and experimental groups, respectively).

Porsolt test is the most popular test for screening of substances with antidepressant activity. The majority of clinically effective antidepressants reduce immobility in Porsolt test by acute injection, but the data on the effects of chronic antidepressant treatment in this test are contradictory [7]. Our data are in line with published reports: acute injection of imipramine reduced the duration of immobility in the forced swimming test, while chronic treatment was ineffective.

Chronic treatment with imipramine reduced the duration of genetically-determined catalepsy in GK rats [5]. The sensitivity of catalepsy reaction to chronic imipramine treatment in NPK mice is similar to the reaction observed in GK rats. Hence, the models of hereditary predisposition to cataleptic reaction in NPK mice and GK rats are similar not only by behavioral manifestations, but also by the effect of imipramine on the intensity of catalepsy.

Hereditarily determined cataleptic reaction in NPK mice is a more convenient form of behavior for studies of antidepressant effects than forced swimming test, because it is sensitive to chronic imipramine treatment, which corresponds to the time course of clinical effect of antidepressants, manifesting only after long-term treatment [6].

Molecular findings and data on functional activity are in line with modern concepts of desensitization of presynaptic 5-HT_{1A} autoreceptors of the midbrain, the postsynaptic receptors of this type remaining unchanged under the effect of chronic antidepressant treatment [6]. The value of NPK mice as the model for studies of the role of 5-HT_{1A} receptors is also explained by the close linkage between the gene regulating predisposition to catalepsy and 5-HT_{1A} receptor gene [3]. Hence, NPK mice can become a new promising model for studies of the participation of 5-HT_{1A} type serotonin receptors in the mechanisms of antidepressant effect.

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