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Effects of dihydropyrimidinase-related protein 2 on anxiety level in rats

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The article concerns studies on molecular mechanisms of anxiety. The studies were carried out on Wistar male rats, through the application of an open field test and an elevated plus-maze model. On the audiogenic stress model, stress-resistant rats were chosen for the following studies. The animals were divided into 3 groups: 1) intact group, 2) control group – inactive dihydropyrimidinase related protein 2 (DRP2), and 3) experimental group – DRP2. Preparations (1 mg/mL, 10 µL) were administered into the brain lateral ventricle of etherized rats 24 h prior to behavioral studies. The results of the studies carried out on the open field test revealed a decline in the number of rearings ($p<0.01$), the total duration of grooming ($p<0.001$), the number of crossed squares ($p<0.01$), and an upregulation of the total duration of freezing in the experimental group ($p<0.01$) relative to the control animals. The results obtained in the elevated plus-maze model showed a sharp decline in the number of rearings ($p<0.001$), total duration of grooming ($p<0.001$), a number of crossed squares ($p<0.001$), a three-fold increase of total duration of freezing time ($p<0.001$) in the closed branch and a prominent, more than four-time decrease in the number of hangings in the experimental animals ($p<0.001$) relative to the control ones. A general conclusion concerning the anxiogenic effects of intra-cerebral administration of DRP2 on animal behavior is put forward.

Keywords: *Male Wistar rats, dihydropyrimidinase related protein 2, open field test, elevated plus-maze, anxiogenic effect*

INTRODUCTION

Nowadays, the problem of the underlying molecular mechanisms of anxiety is still far from its complete clarity. Along with this, it should be noted that this problem has both fundamental and practical importance – evaluation of the emotional status of airport operators and the fatigue of workers on factory conveyor lines. Earlier, we revealed changes in the level of dihydropyrimidinase-related protein 2 (DRP2) in the platelets and saliva and natural anti-DRP2 autoantibodies (Avrameas, 1991; Poletayev, 1995) in the serum of the patients, feeling anxiety on the day of their surgery and having increased levels of the stress hormone cortisol in their serum (Guliyeva, Mekhtiev, 2023). DRP2 is a serotonin-modulating protein known to facilitate axonal outgrowth of the brain neurons (Goshima et al., 1995; Inagaki et al., 2001). However, additional studies are required to clarify the character of such changes in terms of whether they reflect an organism's adaptation to stressful conditions or, conversely, if they are indicative of processes of disadaptation or exhaustion of internal resources. The goal of the present study was to reveal the effect of intra-cerebral administration of DRP2 on the level of anxiety in two behavioral models on rats.

MATERIALS AND METHODS

DRP2 was purified from cow brains through the application of biochemical procedures as

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described earlier (Gaisina et al., 2022). The brains were homogenized in the extracting buffer containing 0.05 M phosphate buffer (pH 7.2), 0.3 M NaCl, 5 mM EDTA and 0.1% Triton X-100 in a volume ratio of tissue and buffer as 1:4. The main stages of fractionations were as follows: 1) protein partial precipitation by ammonium sulfate under the final concentration of 40%, 2) gel-chromatography on the column (3 x 60 cm) of Sephadex G-150, 3) exposure to the effect of 40 mM deionized EDTA throughout the night on the end-to-end shaker, 4) mixture of proteins with EDTA was subjected to isoelectric focusing on a flat-bed gel "Ultrogel" (LKB, Sweden) with the application of ampholines of narrow pH range (pH 4-6; LKB, Sweden). After ending isoelectric focusing, 1 cm width gel strips were collected, pH values were measured in each gel strip and the fraction with a pH value that was equal to the *pI* value of DRP2, was eluted from the gel and analyzed in SDS electrophoresis with protein standards. The process of fractionation and selection of the immune-positive protein fractions was realized under the screening control by the indirect ELISA-test with the application of anti-SMAP polyclonal immunoglobulins (Gaisina et al., 2022).

The behavioral studies were carried out on Wistar male rats of 180-220 g body mass. First, the animals were subjected to audiogenic stress of 90-120 dB strength and only stress-resistant animals were selected for conducting the following series of studies. All stress-resistant rats were divided into 2 groups: 1) intact group, 2) control group – administration of inactive DRP2 (water bath at 60°C, 35 min), and 2) experimental group – administration of active DRP2. The preparations were administered into the brain left lateral ventricle of the anesthetized rats (natrium etaminali 6 mg to 100 g of body mass) at a dose of 1 mg/mL and volume 10 µL, in buffered saline (pH 7.3), at a low speed for 1 min. Behavioral experiments on both models were undertaken 24 h after the administration of the preparation.

The first series of experiments were carried out on the open-field model. This model allows evaluation of both exploratory activity and the anxiety level of the rats. The animals were placed at the center of the experimental box, one at a time, for 1 min under dark conditions and thereafter for 3 min under illuminated conditions (lamp of 150 W power hung at a height of 1 m beyond the floor of the experimental box). The total duration of grooming and freezing acts and the number of rearing acts and crossed squares separately for dark and illuminated conditions were measured.

The second series of experiments was carried out on the elevated plus-maze. This model is a worldwide-accepted model for measuring anxiety levels in rats. The animals, one at a time, were put in the center of the maze and the total duration of freezing and grooming and the numbers of rearing, hanging down, and crossing squares in the closed and open arms of the maze during the 5-minute test were fixed. The average values were calculated for each group and analyzed using the Student's t-criterion.

RESULTS AND DISCUSSION

The results of the experiments undertaken on the open field test demonstrated significant changes in behavioral indices in the experimental group relative to the control group. In particular, under dark conditions, the number of rearings of the experimental animals was less than that of the control ones (1.1±0.5 vs. 2.3±0.2, $p<0.05$), the total freezing time was much higher than that of the controls (18.4±6 sec vs. 0 sec, $p<0.01$) and the number of crossed squares of the experimental group was three times lower than the control values (13.3±1.7 vs. 48.7±2.7, $p<0.001$). Moreover, under the illuminated conditions in the open field test, the differences between the behavioral indices of the experimental and control groups were much sharper than those under dark conditions. In particular, the number of rearing acts was about 4 times lower than in the control group (1.6±0.6 vs. 5.8±1.3, $p<0.01$; Fig.1), total duration of grooming acts was more than three times lower than the values of the control animals (16.7±6.4 sec vs. 57.3±2.9 sec, $p<0.001$; Fig.2), total time of freezing exceeded two times this level of the control rats (127±17.4 sec vs. 64.3±6.6 sec, $p<0.01$; Fig.3), and a number of crossed squares in the experimental group was drastically lower than this index in the control group (29.9±6.9 vs. 49.9±2.3, $p<0.05$; Fig.4).

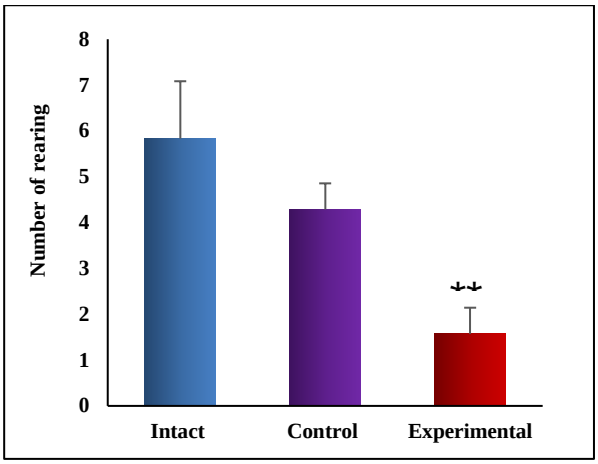

Fig. 1. The number of rearings of the rats in the open field test under illuminated conditions.
** - p<0.01.

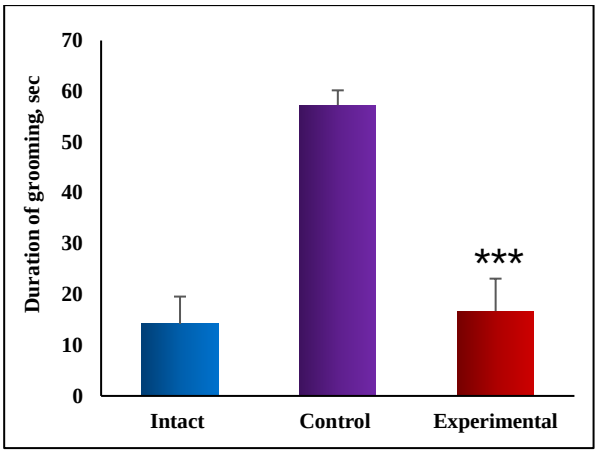

Fig. 2. The total duration of grooming acts in the open field test under illuminated conditions.
*** - p<0.001.

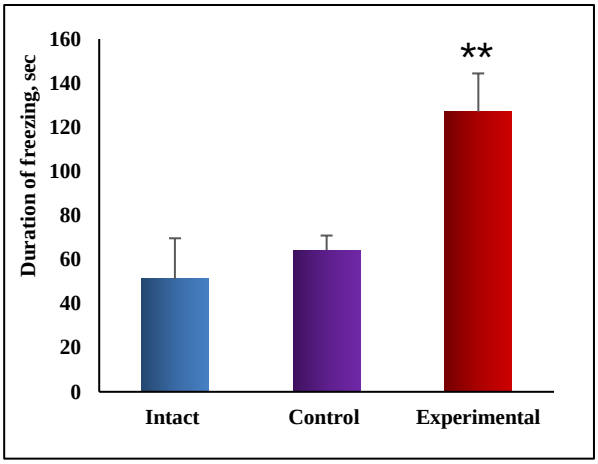

Fig. 3. The total duration of freezing in the open field test under illuminated conditions.
** - p<0.01.

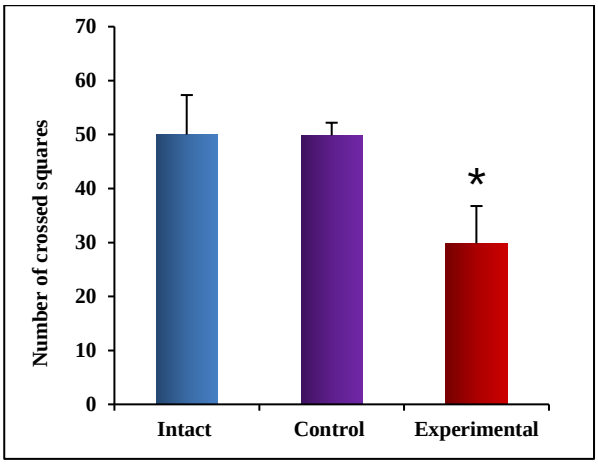

Fig. 4. The number of crossed squares in the open field test under illuminated conditions.
* - p<0.05.

The results of the experiments in the elevated plus-maze model as well revealed significant changes in behavior under the effect of DRP2 intracerebral administration. In particular, in the animals of the experimental group, a sharp decline in the number of rearing acts (1.3±0.5 vs. 13.7±0.6, p<0.001; Fig.5), of the total duration of grooming acts (2.5±1.7 sec vs. 37.3±3.5 sec, p<0.001; Fig.6), of the number of crossed squares (6.2±1.5 vs. 27.7±1.0, p<0.001; Fig.7) in the closed branch of the elevated plus-maze relative to the control values were observed. At the same time, a three-fold increase in the total duration of freezing time in the closed arms in the experimental group relative to the control group (226.7±14 sec vs. 66.3±2.8 sec, p<0.001; Fig.8) was noted.

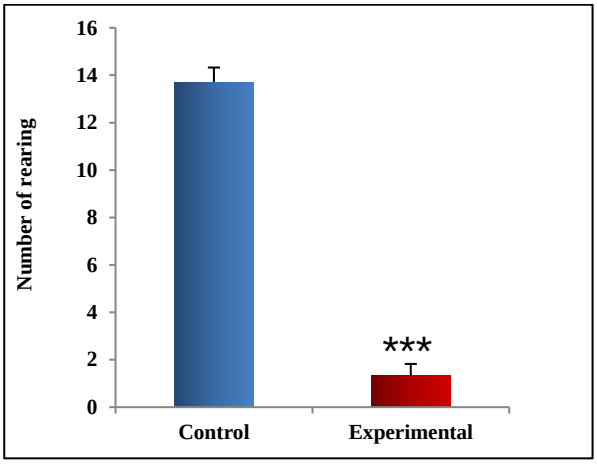

Fig. 5. The number of rearings of the rats in the closed arms of the elevated plus-maze model.
*** - p<0.001.

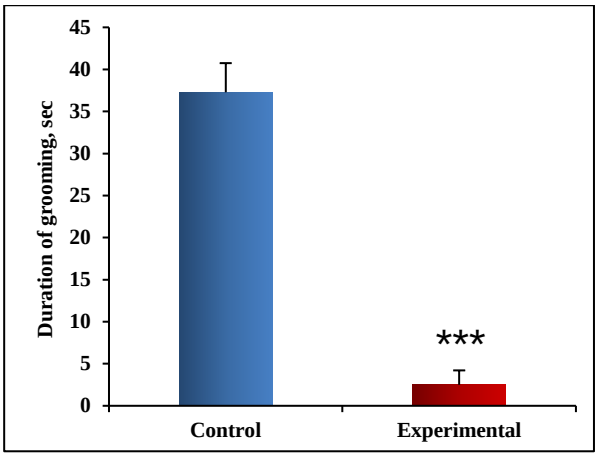

Fig. 6. Total duration of grooming acts of the rats in the closed arms of the elevated plus-maze model.
*** - p<0.001.

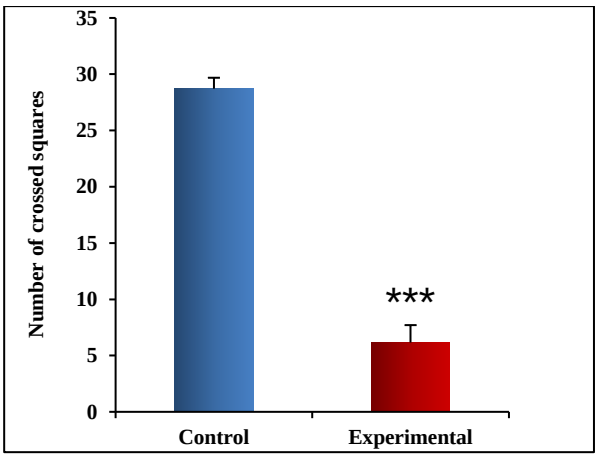

Fig. 7. The number of crossed squares of the rats in the closed arms of the elevated plus-maze model.
*** - p<0.001.

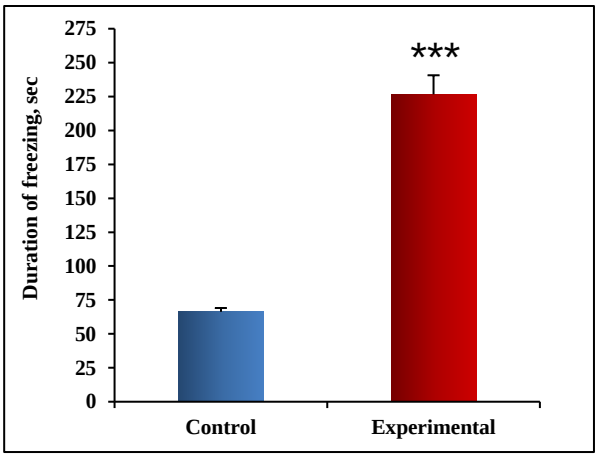

Fig. 8. The total duration of freezing acts of the rats in the closed arms of the elevated plus-maze model.
*** - p<0.001.

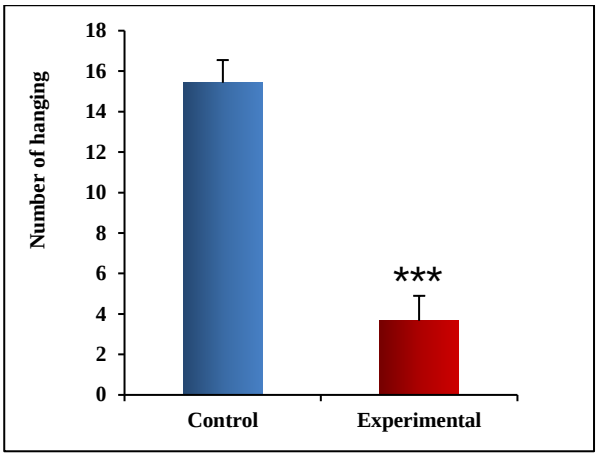

Fig. 9. The number of hangings of the rats in the elevated plus-maze model.
*** - p<0.001.

Furthermore, a number of hangings drastically declined more than four times in the experimental animals relative to the control ones (3.6±1.2 vs. 15.4±1.1, $p<0.001$; Fig.9). There was no difference in the number of crossed squares between the experimental and control groups (4.7±0.2 vs. 5.6±0.5, $p>0.05$).

On the whole, the results obtained from the studies on the rats in two behavioral models designed for analysis of excitation level allowed to reveal significant changes in animal behavior under the effects of intra-cerebral administration of DRP2. Both the results of the open field test – the decline of the number of rearing acts, the total duration of grooming, the number of crossed squares, and the upregulation of the total duration of freezing in the experimental group – and the results of elevated plus-maze model – the sharp decline of a number of rearing acts, of the total duration of grooming acts, of a number of crossed squares, a three-fold increase of a number of total duration of freezing time in the closed branch and prominent, more than four times decrease of the number of hanging in the experimental animals relative to the control ones – give grounds to coming to a general conclusion concerning anxiogenic effects of intracerebral administration of DRP2 on animal behavior.

In terms of the results obtained on rat models of behavioral anxiety, the results by analyzing blood and saliva samples from patients experiencing anxiety on the day of surgery become understandable **(**Guliyeva, Mekhtiev,

2023). The upregulation of DRP2 in the platelets of the excited persons reflects its upregulation in their brain cortex (Collins et al., 2013; Elliott, Kent, 1989), whereas the upregulation of natural anti-DRP2 autoantibodies in their blood serum, according to our own data (Hasanova, 2022), indicates a relative upregulation of DRP2 in subcortical structures. Based on the results we got on the fish (Mekhtiev et al., 2017) and on the rats (Allahverdiyeva et al., 2019), upregulation of DRP2 in the brain structures and generally throughout the body underlies engagement of the adaptation processes, promoting mitigation of the negative effects of adverse factors of different origins on the organism.

CONCLUSION

The results obtained both from the open field test and the elevated plus maze indicate the anxiogenic effects of DRP2 on rat behavior. These results strongly coincide with the results obtained earlier on the patients feeling anxiety on the day of their surgery.

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