Ethological Analysis of the Effects of Fluoglyzine on Mice Exposed to Chronic Social Stress

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Ethological study of a new agent fluoglyzine (fluoxetine analog) was carried out. Porsolt test revealed a positive antidepressant effect of fluoglyzine in mice with pronounced depression-like state. This effect was more pronounced than the effect of fluoxetine. Both drugs effectively improved communicative activity of experimental animals. Anxiety tests showed no anxiolytic effects of fluoglyzine and fluoxetine.

Key Words: *depression-like state in mice; fluoglyzine; fluoxetine; antidepressant effect*

Despite a wide spectrum of drugs used now for the treatment of depression psychopathology, the need for new long-acting low-dose selective antidepressants without side effects remains a pressing problem. Fluoglyzine, a new analog of well-known antidepressant fluoxetine, was synthesized at the Institute of Technological Chemistry, Ural Division of Russian Academy of Sciences by a group headed by Corresponding Member of Russian Academy of Sciences A. G. Tolstikov. Fluoglyzine is a complex drug consisting of fluoxetine and glycyrrhizic acid. Preliminary experiments on intact random-bred mice showed a 20-fold lower toxicity of fluoglyzine in comparison with fluoxetine; antidepressant effect was observed after administration of fluoglyzine in a dose 17.6-fold lower than the dose of fluoxetine (reference agent). Fluoglyzine in a dose of 10 mg/kg increased activity of mice in the forced swimming test by 30%, in doses of 25 and 15 mg/kg by 16%.

We compared the antidepressant and anxiolytic effects of fluoglyzine and fluoxetine in mice with anxious depressive syndrome developed under conditions of chronic social conflict. It was previously shown that male mice with long-term experience of social defeats in intraspecies confrontations with stronger and more aggressive opponents develop behavioral deficiency, their stress reactivity and body weight decrease, they develop anxiety and other symptoms typical of depression-like state [6,7]. Chronic anxiety and fear are regarded as etiological emotiogenic factors causing the development of depression in these animals, similarly as in humans. Some traditional anxiolytics, e.g. 5-HT_{1A} serotonin receptor agonists ipsapirone and buspirone, exhibit a positive effect under these conditions (if injected chronically) [1].

We compared the effects of fluoglyzine and fluoxetine on the status and behavior of mice with preformed anxious depressive syndrome using routine behavioral tests for evaluating depression (Porsolt test [9]), anxiety (elevated plus-maze [11] and open field), and communicative activity (wall test [5]).

MATERIALS AND METHODS

Experiments were carried out on adult (2.5-3 months) male C57BL/6J mice (24-26 g), bred and kept under standard vivarium conditions at the Institute of Cytology and Genetics. The mice were placed into small experimental cages divided with a transparent wall with holes, one animal per compartment. During long-

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term (20 days) daily male-male confrontations performed during the second half of the day (15.00-17.00) the wall was removed for 10 min [5]. In case of intensive (more than 3 min) attacks of aggressive males the wall was placed back. As a result, males with experience of long-term social defeats (victims) were selected, which developed anxious depressive syndrome under the effect of chronic social stress.

Starting from day 21, all victims were divided into 3 groups. Group 1 mice received fluoglyzine orally in a dose of 15 mg/kg for 14 days, group 2 received 15 mg/kg fluoxetine (Nikomed), and group 3 mice received vehicle (water with Twin-80, group 3). The confrontations were stopped after the start of treatment, but all victims remained in the same cages with aggressive opponents behind the wall. All animals were tested in 4 tests (1 test per day) during the treatment.

The elevated plus-maze (50 cm above the floor) consisted of 2 open and 2 closed (with walls on three sides) arms. During the test the animal was placed into the center of the maze with its nose directed into the closed arm. The time spent in open and closed arms and in the center was recorded over 5 min (the data are presented as the percent ratio of the time spent in each part of the maze to the total duration of testing). The number of excursions into open arms, center, and closed arms was expressed (like the time) in percents. The total number of excursions into the open arms and center and excursions into the closed arms of the maze, the number of peeping from closed arms, the number of transitions from one closed arm into the other, and number of peeping under the maze were recorded.

In the Porsolt test the mouse was placed under unavoidable conditions: a glass vessel (diameter 12 cm, height 20 cm, volume 1 liter) filled with water (25±1°C) to a level of 9 cm. Duration of active and passive swimming (drift and complete immobility in water) was recorded over 5-min test. The mouse was considered motionless, if it lay in water without moving. During drive the mouse ventured just weak forced movements with one or both hind paws in order to keep its head above the water surface.

In the open field test the mouse was put into the center of a square area (80×80 cm) divided into 10×10 cm squares, after which a 60 W lamp positioned at the height of 1 m was switched on and the number of crossed squares, latency of the first running from the center of the field, number and duration of rearings,

TABLE 1. Behavioral Parameters of Depressive Male Mice after Chronic Treatment with Drugs, Evaluated in Porsolt, Open Field, and Elevated Plus-Maze Tests

Test; parameter	Control (n=12)	Fluoxetine (n=10)	Fluoglyzine (n=12)
Porsolt test			
duration of passive swimming, sec	173.0±7.1	148.2±11.5	135.8±12.9*
latency, sec	71.5±5.5	61.0±7.4	72.3±7.8
Open field test			
latency, sec	55.8±23.9	55.1±28.5	47.2±23.4
number of crossed squares	50.2±7.9	58±16	50.8±10.6
grooming	5.0±0.8	6.5±1.2	6.1±1.4
duration of grooming, sec	14.4±2.4	21.8±3.9	16.6±3.9
rearing	6.1±1.3	8.5±1.8	9.0±2.1
duration of rearing, sec	6.0±1.6	14.0±3.4*	10.0±2.5
Elevated plus-maze test			
number of excursions into open arms, %	6.9±2.2	4.6±1.9	4.4±1.8
time spent in open arms, %	1.9±0.7	0.4±0.2	0.3±0.1
number of excursions into the center, %	43.6±1.6	41.4±4.9	45.3±1.0
time spent in the center, %	7.2±1.5	6.4±1.1	8.4±1.3
number of entries into closed arms, %	49.5±3.6	54.1±5.7	50.3±2.2
time spent in closed arms, %	90.9±2.1	93.1±1.2	91.3±1.3
total number of entries/exits	11.9±2.2	8.0±1.6	10.5±1.9
peeping out	6.0±0.9	9.2±2.2	5.0±0.6
transitions	1.7±0.6	0.7±0.4	1.8±0.7
peeping down	2.0±0.8	0.8±0.4	2.0±0.5

Note. *p<0.05 compared to the control (solvent).

and number and duration of grooming acts were recorded over 5 min.

In the wall test [5] the behavioral parameters of victims near the wall dividing the males in its reaction to known partner were recorded over 5 min: number of approaches to the wall and the time spent near the wall, when the victim reacted to another animal in the other compartment of the cage, sniffing and touching the wall with forepaws or with the nose. Then the known opponent was replaced with intact male and the same parameters were evaluated in the reaction to unknown partner (also over 5 min).

The data were statistically processed using nonparametric Wilcoxon—Mann—Whitney's *U* test using standard STATISTICA software.

RESULTS

Chronic treatment with fluoglyzine decreased the duration of passive swimming ($p<0.04$) compared to control animals (Table 1). This effect of fluoglyzine was more pronounced than that of the classical antidepressant fluoxetine. The effect of fluoxetine was similar, but negligible ($p<0.09$). Since Porsolt test is sensitive to the effects of antidepressants [2,9,12] and is used for evaluating depression in experimental animals [3], we can speak about antidepressant effects of the studied drugs on victims. It should be noted that fluoglyzine was more effective in this test.

Chronic treatment with fluoglyzine and fluoxetine virtually did not modify the behavior of victims in the elevated plus-maze test (Table 1). As this test is widely used for evaluating anxiety in rats and mice [8,11], we conclude that none of these drugs had anxiolytic effect, at least under our experimental conditions and with the duration of treatment used in our study.

Fluoglyzine virtually did nor modify motor and exploratory activities of mice in the open field test (number of crossed squares, number and duration of rearings, which were the same in treated animals and mice receiving the solvent). In contrast to fluoglyzine, fluoxetine prolonged the time of rearing (Table 1),

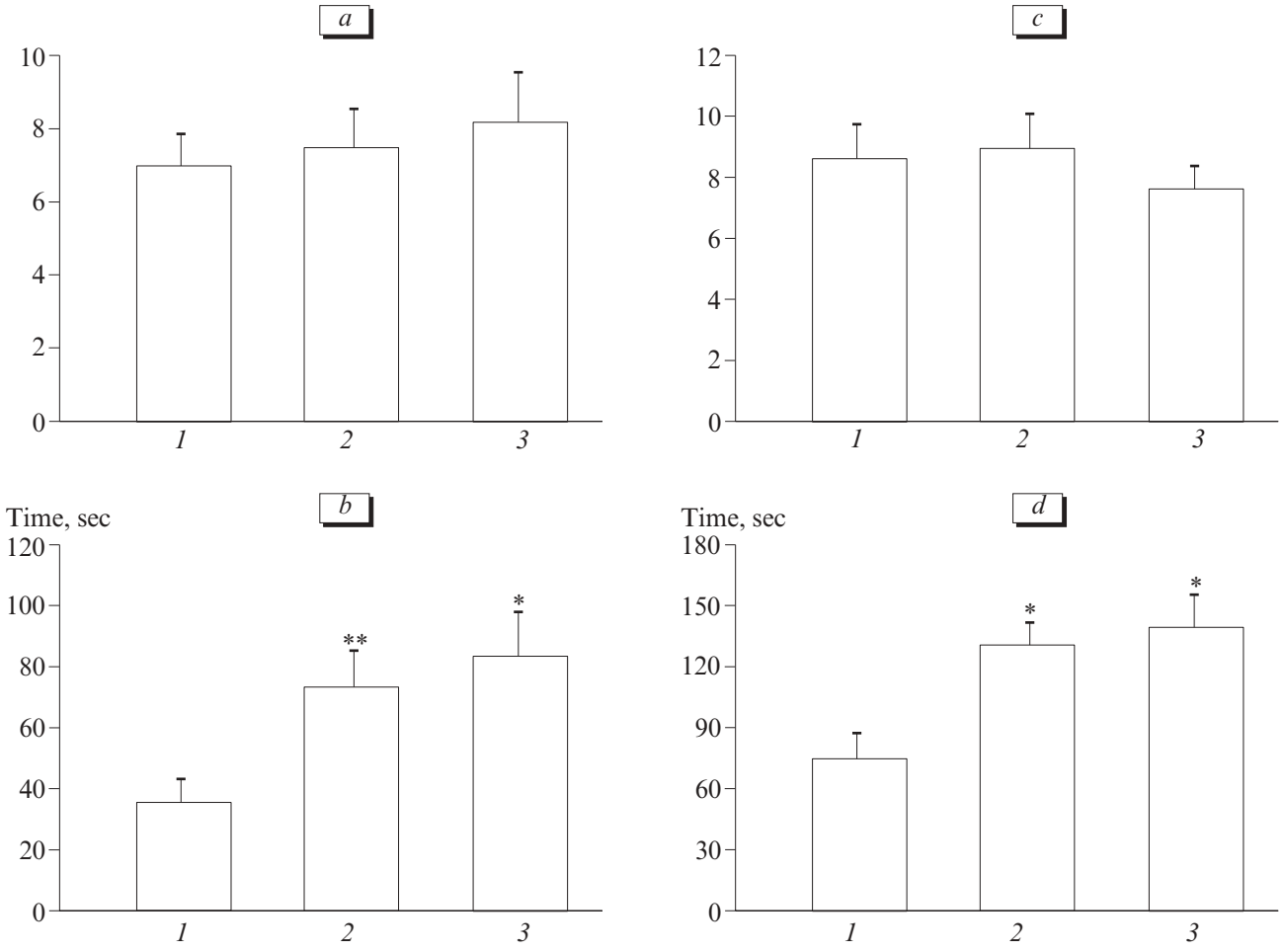

Fig. 1. Changes in the behavior of mice chronically treated with the solvent (*1*), fluoglyzine (*2*), and fluoxetine (*3*) in reactions to a known (*a*, *b*) and unknown (*c*, *d*) partners in the wall test. *a*, *c*) number of approaches to the wall; *b*, *d*) time spent near the wall. **p*<0.01, ***p*<0.05 compared to the control (solvent).

which can reflect increased exploratory activity of experimental animals. This test, together with the elevated plus-maze test is used for evaluation of anxiety in animals [4,10]. Hence, the absence of significant differences between the mice receiving fluoglyzine and solvent attests to the absence of anxiolytic effects of fluoglyzine.

On the other hand, pronounced positive effect of fluoglyzine was observed in the wall test evaluating communicative activity of animals by their reaction to the partner in the contralateral compartment of the cage (Fig. 1). Chronic treatment with fluoglyzine 2-fold increased behavioral activity of mice near the wall ($p<0.02$). Replacement of aggressive male with intact male far increased victim activity (Fig. 1): victims treated with fluoglyzine spent more time near the wall compared to victims receiving the solvent ($p<0.003$). Fluoxetine produced a similar effect: the behavioral reaction of victims to both aggressive and intact males increased after chronic treatment with this drug ($p<0.007$ and $p<0.005$, respectively). Hence, this test demonstrated positive effects of fluoglyzine and fluoxetine, which improved communicative activity of depressive animals and their interest to the partner. It is noteworthy that the effects of the test drug were recorded even under conditions of persisting negative psychogenic exposure (sensory contact between victims and aggressive males).

Coincidence of the effects of a new chemical agent fluoglyzine with those of the reference antidepressant fluoxetine in our experiment, that is, in animals with formed anxious depressive syndrome, allows us to recommend fluoglyzine as a new agent with potential antidepressant effects and with positive effect on the communicative activity of animals.

Thus, new chemical agent fluoglyzine administered chronically to depression-like mice exhibits more pronounced antidepressant effects than the classical antidepressant fluoxetine. Both fluoglyzine and fluoxetine had a universally directed effect on communicative activity of depressive animals under conditions of persisting psychopathogenic exposure. Neither fluoglyzine, nor fluoxetine modulated motor and exploratory activities and showed no anxiolytic effects.

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