EXPERIMENTAL BIOLOGY

Behavioral Effect of 1A-Serotonin Receptor Agonist Ipsapirone in Mice Previously Defeated in Male-Male Encounters

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The effect of 1A-serotonin receptor agonist ipsapirone (3 mg/kg) on mouse behavior was studied in the "wall" and Porsolt tests. The effects of the test drug were compared in intact animals and mice previously defeated in 20 intermale encounters (victims). Ipsapirone was ineffective in victims and effective in intact mice in the wall test. In the Porsolt test the drug prolonged stupor in victims.

Key Words: *mice; intermale encounters; ipsapirone; behavior*

1A-serotonin receptor agonists (1A-SR), including ipsapirone, are now often used as anxiolytics [7] and antidepressants [12]. They produce no side effects and therefore are preferable to traditionally used benzodiazepines [8]. With therapeutic purposes, ipsapirone is administered in long-term courses [8]. In animal experiments its effect can be detected as soon as 30 min after a single injection [1,7], but this effect is not always anxiolytic [7].

These contradictory data can be explained by the fact that experiments are carried out on animals with initially different status (intact or with some disease). It was interesting to investigate the effect of ipsapirone on animals which experienced long social stress in intermale encounters (victims); after consecutive defeats for 20 days these animals develop depressive-like state [10,11]. Apart from neurochemical and endocrine disorders [10], experimental depression in the victims is paralleled by manifestations of behavioral deficiency in many tests [3,10]. For example, freezing in the Porsolt test (used for screening of antidepressants) was prolonged in victims compared to intact animals [5, 14,15]. Moreover, stress caused pronounced anxiety in mice at the early stages of depression. This anxiety persisted for a long time [3] and was observed in mice in the "wall", elevated plus-maze, or open field tests [10]. This suggests that the effect of 1A-SR ipsapirone is different in victims and intact mice.

We compared the behavior of victims experienced defeats in 20 intermale encounters and intact nonstressed mice in the «wall» and Porsolt tests after a single injection of ipsapirone.

MATERIALS AND METHODS

Adult (2.5-3-month) male C57Bl/6J mice (24-26 g) from Stolbovaya Breeding Center, Moscow region, were used. In order to remove the stress caused by transportation, the animals were put in 36×23×12 cm cages, 6-8 animals per cage, and kept under standard vivarium conditions at 12-h day/night regimen, and free access to food and water for 2 weeks. After this the animals were put into experimental cages (28×14× 10 cm) divided into 2 equal compartments with a transparent wall with holes, one mouse per compartment. These conditions allowed the animals to see, hear, and

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smell each other (sensory contact) but allowed no physical contact.

After 2-day adaptation to new conditions and sensory acquaintance the animals were tested in intermale encounters in the second half of the day (15.00-17.00). The cover of the cage was replaced with transparent organic glass for observations and after 5 min (period of animal activation and adaptation to new illumination) the wall was removed for 10 min, which led to antagonistic interaction (intermale encounter). Defeats in the first tests (during 3 days) experienced by one male in each pair were then fixed in repeated encounters with aggressive partners. To this end, a defeated male was put after the encounter into a new cage with another aggressive male behind the wall. Aggressive males remained in their compartments over the entire experiment. If intensive attacks of aggressive partners during antagonistic encounters lasted for more than 3 min, encounters were stopped by putting a wall between the mice. As a result, a group of mice with 20-day experience of social defeat (victims) was selected.

Thirty minutes before behavioral tests some victims were intraperitoneally injected with ipsapirone (3 mg/kg, Tropenwerke) and others with 0.9% NaCl (solvent). The efficiency of this dose was demonstrated previously [1]. Intact controls received similar injections of ipsapirone or 0.9% NaCl. Controls were put into individual cages for 5 days; this removed the experience of group interactions and did not lead to social isolation.

All animals were tested in 2 behavioral tests: "wall" test [9] and Porsolt test [14]. The "wall" test allows quantitative evaluation of animal communication by behavioral reaction to the partner in the neighboring compartment of the common cage behind the transparent wall with holes. Testing included registration of behavioral reaction to a known partner: number of approaches to the wall during 5 min test and the total time spent near the wall by each mouse, when animals reacted to the individual in the neighboring compartment of the cage by sniffing and touching the wall with the front paws or nose. Then the known partner was replaced with an intact male, which was kept in a group, and the same parameters were assessed in the reaction to an unknown partner.

Porsolt test is sensitive to antidepressants [5,14, 15] and is used for evaluating the depressive status of animals. Each mouse was placed under unavoidable conditions: a 1-liter glass (12 cm in diameter, 20 cm high) filled with water (25±1°C, 9 cm high). Total time and the number of immobility episodes in the water were evaluated during 5 min.

Behavioral parameters were recorded by an Etograf device fixing the incidence and duration of a behavioral act.

The data were compared using Mann—Whitney nonparametrical *U* test and Statistica for Windows software.

RESULTS

Ipsapirone significantly modified the behavior of intact mice in the "wall" test with to both known and unknown partners (Table 1). In both cases the drug reduced the number of approaches to the wall and the time spent near it. As mouse behavior in this test largely depended on the anxiety state [3], the effect of ipsapirone can be regarded as anxiogenic. Previously a similar anxiogenic effect of ipsapirone on intact C57B1/6J mice was demonstrated in another anxiety test, elevated plus-maze test [1] commonly used for evaluating anxiety in rats and mice [7].

Replacement of a known animal behind the wall with an unknown one significantly changed the reac-

TABLE 1. Behavior of Experimental Animals in the Wall and Porsolt Tests (*M*±*m*)

Parameter	Control		Victims	
	0.9% NaCl	ipsapirone	0.9% NaCl	ipsapirone
Wall test				
Reaction to a known partner				
number of approaches	12.67±1.61	6.54±1.95*	4.50±0.98*	3.50±1.05*
duration, sec	46.58±9.96	20.91±7.57**	13.38±3.62**	5.75±1.56*++
Reaction to an unknown partner				
number of approaches	17.75±2.21	10.55±2.57**	5.75±1.47*	5.25±1.75*
duration, sec	107.42±16.86	40.00±12.81*	25.88±9.26*	23.50±8.20*
Porsolt test				
Duration of immobility, sec	129.00±13.82	119.64±7.66	149.13±8.31	173.50±12.51**+

Note. *$p<0.01$, **$p<0.05$ *vs.* control (0.9% NaCl), +$p<0.01$, ++$p<0.05$ *vs.* control (ipsapirone).

TABLE 2. Comparison of Reactions to a Known and Unknown Partners in the Wall Test

Parameter	Control		Victims	
	0.9% NaCl (*n*=12)	ipsapirone (*n*=11)	0.9% NaCl (*n*=8)	ipsapirone (*n*=8)
Number of approaches				
U	38.0	39.0	28.5	25.5
p	<0.05	D. n.	D. n.	D. n.
Duration, sec				
U	25.0	33.5	23.0	17.5
p	<0.01	D. n.	D. n.	D. n.

Note. D. n.: the difference is negligible.

tion of intact mice injected with 0.9% NaCl: the number of approaches to the wall and time spent near it increased (Table 2). Increased activity of mice in response to an unknown mouse can be due to increase of their communication, as was suggested previously [9]. However this reaction was not observed in the group of controls treated with ipsapirone (Table 2), which can be attributed to the anxiogenic effect of the drug.

The victims differed significantly from intact animals by their reaction to both known and unknown partners. They more rarely approached the wall and spent less time near it. In other words, the victims avoided contacts with mice behind the wall. Similar negative effect of numerous defeats on the behavior of victims in the "wall" test was demonstrated previously [3,10,11]. Presumably the victims develop manifest probably pathological anxiety under these conditions of social stress [3,4]. Ipsapirone little affected victim behavior in the "wall" test (Table 1). However a weak (negligible) anxiogenic effect was seen: the time spent by the victims near the wall decreased even more, differing significantly from that of intact mice. Hence, the drug potentiated the negative effect of intermale encounters on victim behavior in the "wall" test.

In Porsolt test ipsapirone did not modify the behavior of control mice: the duration of immobility was the same in both control groups (Table 1). Though the behavior of victims treated with 0.9% NaCl did not differ significantly from that of controls, they stayed motionless slightly longer. After ipsapirone injection the difference between victims and controls became significant (Table 1). Presumably, ipsapirone augmented the status of victims, caused by intermale encounters. This effect can be hardly attributed to ipsapirone effect on motor activity, because, as we saw in the "wall" test, the victims treated with ipsapirone and 0.9% NaCl did not differ by the number of approaches to the wall. It was previously shown that this parameter reflected motor activity of mice [3,10].

Hence, a pronounced effect of ipsapirone was observed in the controls in the wall test, while in the Porsolt test the drug was ineffective in intact mice and modified the behavior of victims. As the same dose of ipsapirone differently affected the behavior of control mice and animals with pathological state, we may conclude that the drug effect is determined primarily by the initial status of the animal. As we showed previously, intact mice and animals experienced defeats in 20 intermale encounters differed in cerebral serotoninergic system activity [2,4]. 1A-SR in the brain are located in dendrites and soma (presynaptic autoreceptors) and in serotoninergic terminals (postsynaptic receptors), and therefore the findings of this study can be explained by different sensitivity of these receptors to ipsapirone in intact mice and victims.

In addition, we believe that the two tests help to differentiate activities of pre- and postsynaptic 1A-SR. This hypothesis is in line with the data demonstrating different involvement of the pre- and postsynaptic 1A-SR in the mechanisms of anxiety and depression [6, 13]. Different participation of pre- and postsynaptic 1A-SR in the mechanisms of anxiety in mice is explained by the use of different behavioral tests [13]. Antidepressant effects of ligands on the pre- and postsynaptic 1A-SR depend on the experimental model of depression [6]. In our experiments the effect of ipsapirone observed in the Porsolt test is due to modulation of postsynaptic 1A-SR, because the drug potentiated the behavioral effect in victims and was ineffective in intact animals. If the drug had affected the presynaptic component of the system, it would have manifested in intact animals as well, because intact mice and victims did not differ by the sensitivity of presynaptic 1A-SR [4]. Presumably the anxiogenic effect of ipsapirone in the "wall" test is mediated by both pre- and postsynaptic 1A-SR, as the drug was effective in intact mice and in effective in victims. This is only one of the possible mechanisms, which should be proven by further studies.

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