Effect of 5-HT$_{2C}$ Receptor Antagonist RS 102221 on Mouse Behavior

E. G. Kuznetsova*, T. G. Amstislavskaya, E. A. Shefer, and N. K. Popova

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Injection of RS 102221 (selective antagonist of serotonin 5-HT$_{2C}$ receptors) in a dose of 2 mg/kg reduced anxiety of mice in the light-darkness test and decreased the amplitude of the startle reflex. RS 102221 in a dose of 1 mg/kg reduced prestimulus inhibition of the startle reflex. No behavioral changes in Porsolt test and motor activity in the open field test were detected. Hence, RS 102221 is characterized by selective anxiolytic activity, and 5-HT$_{2C}$ receptors are involved in the mechanisms of anxiety and startle reflex formation.

Key Words: *5-HT$_{2C}$ receptors; RS 102221; behavior; startle reflex; anxiety*

Anxiety and depression predominate in modern psychotherapeutic practice: almost 50% patients suffer from these conditions [1]. Dysregulation of 2C subtype serotonin receptors can promote the manifestation of specific symptoms of anxiety and depression [6]. 5-HT$_{2C}$ receptors are located postsynaptically and seem to be expressed exclusively in the CNS. The highest density of 5-HT$_{2C}$ receptors is observed in the choroid plexus; high 5-HT$_{2C}$ receptor content was also found in the subthalamic nuclei, hypothalamus, hippocampus, and amygdaloid complex [7]. 5-HT$_{2C}$ receptors are hypothesized to be involved in the regulation of many physiological reactions and behavioral forms, including thermoregulation, nutrition, anxiety, sexual behavior [5]. Considerable affinity of some psychotropic agents used in clinical practice (for example, tricyclic antidepressants) for 5-HT$_{2C}$ receptors underlies the interest to studies of the therapeutic potential of selective high-affinity 5-HT$_{2C}$ ligands [6,9]. RS 102221 is the first selective antagonist of 5-HT$_{2C}$ receptors [4]; it is characterized by nanomolar affinity for human (pKi=8.4) and rat 5-HT$_{2C}$ receptors (pKi=8.5) and by a 100-fold higher selectivity to subtype 2C serotonin receptors than to 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors.

We studied the effect of RS 102221, selective 5-HT$_{2C}$ receptor antagonist, on anxiety, depression, motor activity, and startle reflex in mice.

MATERIALS AND METHODS

Experiments were carried out on male CBA/LacSto mice from vivarium of Institute of Cytology and Genetics (Novosibirsk). The animals were kept 6-10 per cage at natural illumination and free access to water and food. Three days before testing the animals were placed into individual boxes in order to remove the effects of social intercourse. Twenty min before testing the animals were intraperitoneally injected with 5-HT$_{2C}$ receptor antagonist RS 102221 (8-[2,4-dimethoxy-5-(4-trifluoromethylsulfo-amido)phenyl-5-oxophenyl]-1,3,8-triazaspiro-[4,5]-decan-2,4-dion; Tocris Cookson) in doses of 1 and 2 mg/kg. Controls were injected with saline. Intact mice served as an additional control group. The testing was carried in animals aged 2.5 months.

Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk; *Novosibirsk State University. ***Address for correspondence:*** keg182@rambler.ru. E. G. Kuznetsova

The behavior of mice was videorecorded and processed [2].

The number of passages from one compartment into the other and time spent in the light compartment were recorded (5 min) in the light-darkness test in a cage consisting of an open light compartment and a closed dark compartment, connected through a hole in the wall.

In the open field test, the number of crossed squares, number of excursions into the center, and time spent in the center were recorded (5 min).

Startle reflex to acoustic stimulus was studied using an SR-Pilot device (San-Diego Instruments Inc.). Startle reflex can be attenuated (prestimulus inhibition) by offering a weaker acoustic signal before the stimulus [11]. Prestimulus inhibition is used as a quantitative indicator of sensorimotor gating.

The force of the main (standard) acoustic stimulus was 115 dB, duration 40 msec. In order to record the value of prestimulus inhibition, the prestimulus (85 dB, 40 msec) was presented 100 msec before the main stimulus. Noise of 65 dB was constantly maintained in the test chamber. The mouse was left there for 3 min for adaptation, after which 4 solitary and 4 paired acoustic stimuli were presented at 15-sec intervals, alternating the solitary standard stimulus and the prestimulus+standard stimulus pair. The startle amplitude was determined (mean for the group reaction to the main stimulus) and prestimulus inhibition (PSI), which was calculated by the formula:

$$PSI=((S-PS)/S)\times 100\%,$$

where S is startle amplitude in response to solitary stimulus and PS startle amplitude in response to a combination of prestimulus and stimulus.

In Porsolt forced swimming test the mouse was placed in a Plexiglas cube (18×18 cm) filled with water to a level of 10 cm (t^o=25.0±0.5^{o}C). Testing was started after 40 sec; the number of crossed squares and duration of immobility (passive swimming) were recorded over 3 min.

The results were statistically processed using Statistica 6 software. The significance of intergroup differences was evaluated using Mann—Whitney's test, correlation between the signs was evaluated after Spearman.

RESULTS

In the light-darkness test, the mice injected with 2 mg/kg RS 102221 spent 2-fold more time in the light compartment compared to intact animals (Fig. 1). The drug in a dose of 1 mg/kg did not change the time spent in the light compartment, similarly as saline. It was previously noted that RS 102221 in doses of 0.06, 0.25, 1, 4, 8, and 16 mg/kg was inessential for the time spent in the light compartment and for the number of passages between the compartments in the light-darkness test [8]. Active dose in our experiment was 2 mg/kg (in this daily dose this drug increased food consumption and body weight gain in rats [4], which is in line with our data on anxiolytic effect of RS 102221 in a dose of 2 mg/kg). The number of passages from one compartment into the other decreased 3-fold after 2 mg/kg RS 102221 in comparison with control and intact animals. A lower dose of the drug virtually did not modify this parameter. Analysis of correlations showed a significant negative correlation between the number of passages from one compartment into the other and the time spent in the light compartment for animals treated with the drug (for 1 mg/kg: r=-0.70, p<0.05; for 2 mg/kg: r=-0.98, p<0.001). It was hypothesized that RS 102221 reduced the mobility of animals, but evaluation of motor activity in open field test did not confirm this

TABLE 1. Effect of RS 102221 on the Behavior of Male Mice ($M\pm m$)

Parameter	Intact mice	Mice injected with saline	Mice injected with RS 102221	
			1 mg/kg	2 mg/kg
Open field test				
number of squares	45±15	62±14	32±8	34±12
number of excursions into the center	2.4±2.0	1.7±1.1	0.4±0.4	1.7±1.3
time spent in the center, sec	3.9±2.5	1.5±0.9	0.7±0.7	2.9±2.8
Porsolt test				
number of squares	158±11	208±15*	210±15*	168±20
immobility time, sec	92±5	81±5	78±4*	93±6

Note. *p<0.05 compared to intact mice.

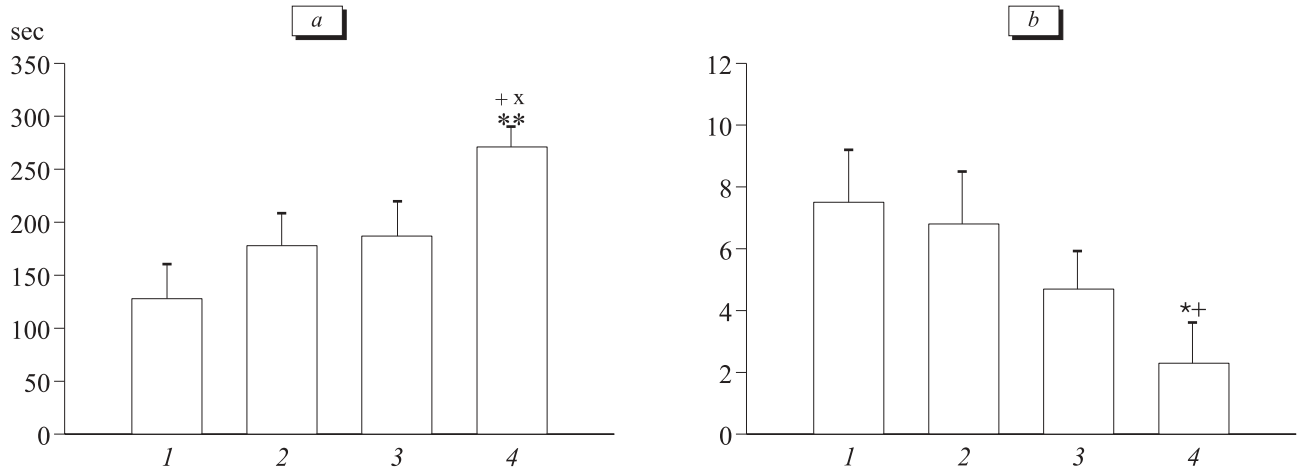

Fig. 1. Effect of RS 102221 on mouse behavior in the light-darkness test. *a*) time spent in light compartment; *b*) number of passages. Here and in Fig. 2: *1*) intact mice; *2*) mice injected with saline; *3*) mice injected with 1 mg/kg RS 102221; *4*) mice injected with 2 mg/kg RS 102221. *$p<0.05$, **$p<0.01$ compared to intact mice, $^{+}p<0.05$ compared to mice injected with saline, $^{x}p<0.05$ compared to mice injected with 1 mg/kg RS 102221.

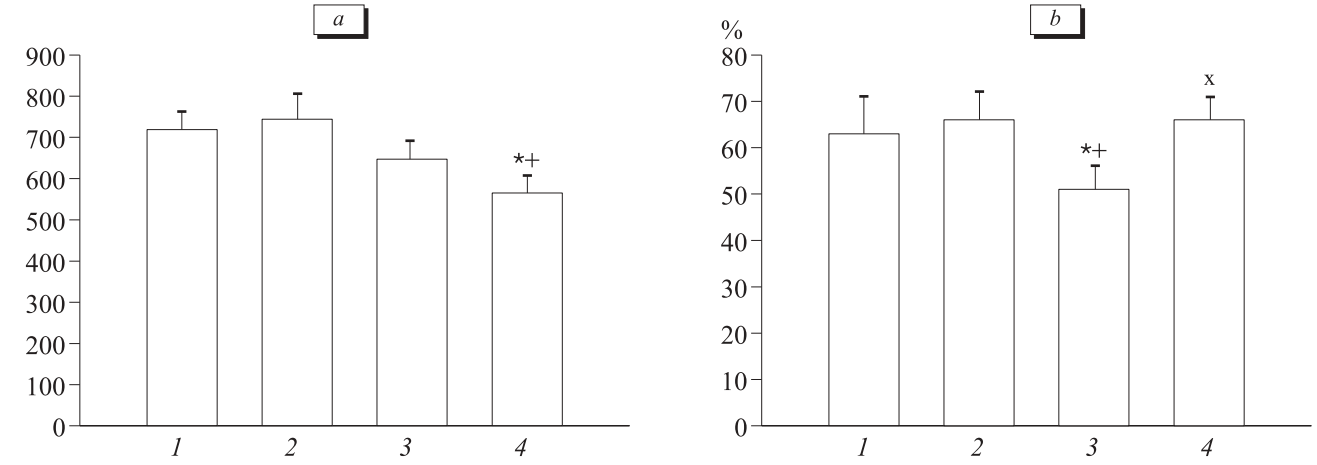

Fig. 2. Effect of RS 102221 on startle reflex of mice. *a*) startle amplitude; *b*) prestimulus inhibition.

hypothesis: the number of crossed squares, similarly as other parameters of open field behavior, were virtually the same in mice treated with the drug and controls (Table 1). Thus, RS 102221 is characterized by selective anxiolytic activity not associated with inhibition of motor activity. Hence, blockade of 5-HT_{2C} receptors leads to reduction of anxiety.

In forced swimming test the mice injected with the drug and saline demonstrated similar behavior, though the duration of immobility (passive swimming) was reduced and the number of crossed squares (indicator of active swimming) increased in animals injected with RS 102221 (1 mg/kg) in comparison with intact animals. As injection of saline also resulted in an increase in the number of crossed squares, it seems that increased activity was a result of injection. Hence, Porsolt test showed that RS 102221 in the studied doses exhibited no antidepressant activity.

RS 102221 in a dose of 2 mg/kg significantly reduced the amplitude of startle reflex (Fig. 2). It was previously shown that 5-HT_2 receptors were involved in the regulation of startle amplitude [3, 10]. Mixed 5-$HT_{2A/2C}$ receptor antagonist ritanserine (0.1 and 0.2 mg/kg) and 5-HT_{2A} receptor antagonist exhibiting also low affinity for 5-HT_{2C} receptors ketanserine (0.5 mg/kg) increased startle amplitude [3]. Our results suggest that blockade of 5-HT_{2A} receptors increase startle amplitude, while blockade of 5-HT_{2C} receptors produces an opposite effect. This is in line with the fact that startle amplitude decreased after the dose of ketanserine was increased to 2 mg/kg [3]. It can be hypothesized that ketanserine in a low dose interacts primarily with 5-HT_{2A} receptors and in a higher doses starts binding to 2C receptors.

A clear-cut attenuation of the prestimulus inhibition was observed after injection of RS 102221

in a dose of 1 mg/kg. It was previously shown that the extent of prestimulus inhibition increased after injection of ketanserine [3]. Blockade of 5-HT_{2C} receptors in this experiment decreased prestimulus inhibition or did not modify this parameter, and we therefore concluded that the increase in prestimulus inhibition was also due to 5-HT_{2A} receptor blockade.

Our data attest to the existence of common mechanisms of regulation of startle amplitude and anxiety, because blockade of 5-HT_{2C} receptors reduced anxiety and decreased the amplitude of startle reflex.

Hence, our findings indicate that blockade of 5-HT_{2C} receptors decreased anxiety, startle amplitude, and prestimulus inhibition deficiency, but is inessential for depression in Porsolt test and motor activity of male mice.

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