Effect of $5HT_{2C}$-Receptor Agonist MK-212 on Blood Corticosterone Level and Behavior in Mice

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A dose-dependent the effect of $5HT_{2C}$-receptor agonist MK-212 on mouse behavior was demonstrated. Intraperitoneal injection of MK-212 in high doses (0.5 and 1.0 mg/kg) increased blood level of corticosterone in mice and reduced their motor activity. In low doses of 0.1 and 0.2 mg/kg, the agonist reduced anxiety, but had no effect on motor activity. It is hypothesized that low doses of MK-212 exhibited anxiolytic activity in mice.

Key Words: *$5HT_{2C}$-receptors; MK-212; behavior; anxiety*

Serotonin $5HT_2$-receptors are divided into three subtypes: $5HT_{2A}$-, $5HT_{2B}$-, and $5HT_{2C}$-receptors. These receptors are positively coupled to phospholipase C via Gq-protein, and their activation leads to the release of inositol-1,4,5-triphosphate and intracellular calcium [2,10]. The first cloned and sequenated genes of $5HT_2$-receptor moiety were those of $5HT_{2C}$-receptors, which initially were erroneously considered as belonging to the $5HT_{1C}$-subtype [2]. $5HT_{2C}$-receptors are located on postsynaptic membranes and are expressed in CNS only. Activation of these receptors depolarizes the neuron. The maximum density of $5HT_{2C}$-receptors was found in the choroid plexus, although significant concentrations of these receptors are characteristic of subthalamic nuclei, hypothalamus, hippocampus, and the amygdale complex [15]. $5HT_{2C}$-receptors are suggested to control many physiological reactions and behavioral patterns including temperature regulation, feeding and sexual behavior, anxiety, *etc.* [4,5,9]. There is a hypothesis on the role of serotonin in the pathogenesis of anxiety, which postulates that inhibition of serotonin neurotransmission produces an anxiolytic effect, while its activation exerts an opposite anxiogenic action [7,12]. The disturbances in regulation of $5HT_{2C}$-receptors located predominantly in the brain can contribute into manifestations of specific anxiety symptoms [9]. MK-212 is a selective agonist to 2C-subtype serotonin receptors. It elevates corticosterone in rat blood [8,13]. Experiments on rats showed that MK-212 (0.5 mg/kg) enhanced anxiety in the light/dark transition test and elevated plus-maze test [12]. In a higher dose of 2 mg/kg, MK-212 also produced an anxiogenic effect in rats placed in the elevated plus-maze [6]. However, some researches reported that agonists of $5HT_{2C}$-receptors (mCPP and RO 60-0175) produced no effect on anxious behavior of mice in the light/dark transition test in a wide dose range and acted as anxiolytics in the elevated plus-maze test [14]. Most anxiety-related action of $5HT_{2C}$-ligands were carried out on rats [7]. Although the distribution of $5HT_{2C}$-receptors in mouse and rat brain is similar [15], in many cases it is difficult to compare the pharmacological data obtained on the anxiety models of both species. Probably, the experimental paradigm provoking stress and despair produce different effects on rats and mice and different brain regions and neuro-

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transmitters can be stimulated in the same test [14]. At present, mice are the most popular objects for genetic studies, so examination of physiological and behavioral effects of neural transmission activation in these experimental animals is gaining in importance.

Our aim was to study the effect of MK-212, an agonist to $5HT_{2C}$-receptors, on anxiety and motor activity of mice and on blood corticosterone level.

MATERIALS AND METHODS

The experiments were carried out on 2.5-month-old male CBA/LacIcg mice from Vivarium of Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences, Novosibirsk). The mice were kept 6-10 per cage under natural illumination regimen and unrestricted food and water diet. Three days before testing, the mice were placed in separate cages to eliminate social effects.

Twenty minutes before testing or decapitation, the mice were intraperitoneally injected with MK-212 (6-chloro-2-[1-piperaxinyl]pyrazine, Tocris Cookson LTD), a selective agonist to $5HT_{2C}$-receptors in doses of 0.1, 0.2, 0.5, and 2.0 mg/kg. The control mice were injected with physiological saline at the same terms. Intact mice were used as additional control. Mouse behavior was videotaped and the data were processed using special software [1].

The light/dark test was performed in a chamber divided into two compartments (27×27×27 cm and 18×27×27 cm) connected with a 6×6 cm hole. The lower (dark) compartment was closed with a lid; the other (light) compartment was illuminated. A mouse was placed into the light compartment near the hole with its snout directed to the entry into the dark compartment. The time spent in the dark compartment (anxiety level) was recorded over 5 min. The increase in this parameter attested to anxiety increase.

The open field test was performed in a plastic chamber 80×80×20 cm and illuminated with a 250 W lamp. A mouse was placed into this chamber near a wall at a special mark. The following parameters were recorded (over 5 min): the number of crossed squares (locomotor activity), the number of rearings (explorative activity), the number of entries into the center and the time spent there (indices of anxiety).

For blood corticosterone assay, the mice were rapidly decapitated, and the blood was centrifuged. The plasma was stored at -20°C. The measurement of plasma corticosterone based on competitive binding of the labeled and non-labeled glucocorticoids with transcortin.

The data were analyzed statistically using Mann—Whitney *U* test and Statistica 6 software.

RESULTS

The experimental mice injected with MK-212 (0.1 and 0.2 mg/kg) spent less time in the dark compartment of the light/dark chamber then the control mice injected with physiological saline (Fig. 1), which indicate moderation of anxiety in experimental mice. The higher doses of MK-212 (0.5 and 1.0 mg/kg) produced no effect on this parameter. These data disagree with the reports on anxiogenic action of the agonists to $5HT_{2C}$-receptors (MK-212 included) in rats [6,12]. At the same time, our data confirm previous observations [14] that stimulation of $5HT_{2C}$-receptors in mice produces an anxiolytic effect. In the cited paper, $5HT_{2C}$-agonists significantly reduced anxiety of Swiss mice in the elevated plus maze, but produced no effect on these mice in the light/dark chamber. Probably, Swiss mice differ from CBA mice used in our study. Specifically, Swiss mice receiving injection of the solvent (distilled water) 55 sec of 5 min testing period spent in the dark compartment. By contrast, control and intact CBA male mice spent 179 and 160 sec in the dark compartment, respectively. Evidently, Swiss mice are characterized by initially lower anxiety level compared to CBA mice. This peculiarity can explain the fact that anxiolytic effect of $5HT_{2C}$-agonists was not revealed in the light/dark test.

The open field test showed that MK-212 produced a dose-dependent effect on locomotion and explorative behavior (Table 1). The preparation in a dose of 0.1 mg/kg increased the number of re-

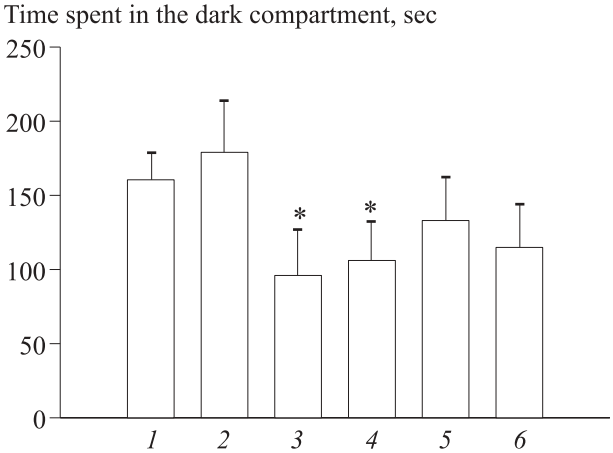

Fig. 1. Effect of MK-212 on mouse behavior in light/dark transition test. *1*) intact mice (n=10); *2*) control mice injected with physiological saline (n=9); *3*) mice injected with 0.1 mg/kg MK-212 (n=9); *4*) mice injected with 0.2 mg/kg MK-212 (n=10); *5*) mice injected with 0.5 mg/kg MK-212 (n=9); *6*) mice injected with 1 mg/kg MK-212 (n=10). *p<0.05 compared to control mice injected with physiological saline.

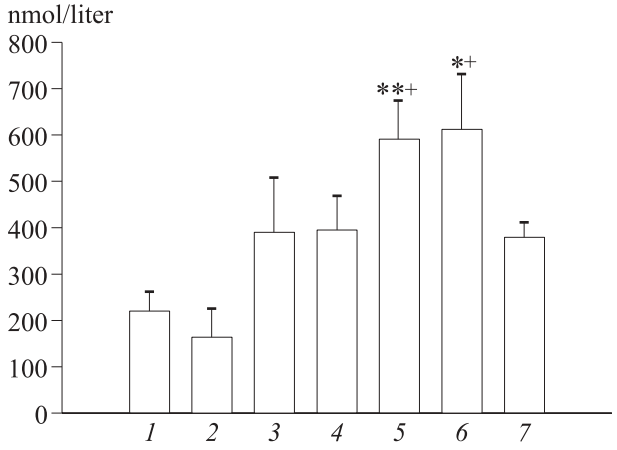

Fig. 2. Effect of MK-212 on plasma corticosterone in mice. *1*) intact mice (n=6); *2*) control mice injected with physiological saline (n=6); *3*) mice injected with 0.1 mg/kg MK-212 (n=8); *4*) mice injected with 0.2 mg/kg MK-212 (n=10); *5*) mice injected with 0.5 mg/kg MK-212 (n=6); *6*) mice injected with 1 mg/kg MK-212 (n=6); *7*) mice injected with 2 mg/kg MK-212 (n=9). *p<0.05, **p<0.01 compared to the intact mice. ^{+}p<0.05 compared to the control mice injected with physiological saline.

arings (p<0.05), while a dose of 0.2 mg/kg produced no behavioral effects. The higher doses of MK-212 inhibited the explorative activity: the dose of 0.5 mg/kg decreased the number of crossed squares (p<0.01), while the dose of 1 mg/kg decreased the number of crossed squares (p<0.01) and the number of rearings (p<0.05). Thus, MK-212 in low doses had no effect on motor activity, but in high doses inhibited it. The lowest examined dose (0.1 mg/kg) increased explorative activity, which agrees with anxiolytic action of MK-212 in this dose. The highest examined dose of MK-212 inhibited explorative activity, which probably relates to general inhibition of locomotion. Experiments on rats showed that low doses of MK-212 produce a selective anxiogenic effect, while high doses of this agonist inhibit locomotion [3,6]. In contrast to rats [6], motor activity of mice is inhibited by MK-212 in far lower doses. Inhibition of motor activity in the open field was documented in rats injected with 4 mg/kg MK-212, while the lower doses of 2 and 1 mg/kg did not affect motor activity. Probably, different sensitivity of mice and rats to MK-212 (and probably, to other $5HT_{2C}$-agonists) revealed in the open-field test reflects interspecies differences. It is unknown whether different sensitivity to MK-212 results from different responses of mice and rats to stress or from the differences of their serotoninergic systems.

The lowest examined doses of MK-212 (0.1 and 0.2 mg/kg) produced no effect on plasma corticosterone (Fig. 2), but higher doses of 0.5 and 1.0 mg/kg elevated it (p<0.05). MK-212 in a dose of 2 mg/kg did not change corticosterone level. In rats, MK-212 elevates blood level of this hormone [8,13] when used in low (1 mg/kg, [13]) or far greater (2 and 10 mg/kg) doses. Elevation of corticosterone results from stimulation of $5HT_{1A}$- and $5HT_{2C}$-receptors [11]. Elevation of plasma corticosterone induced by 8-OH-DPAT (a selective agonist to $5HT_{1A}$-receptors) can be moderated only by the block of $5HT_{1A}$-receptors with their selective antagonists, but not with non-selective serotoninergic preparations. At the same time, the increase of corticosterone induced by stimulation of $5HT_{2C}$-receptors with selective agonist MK-212 can be moderated by antagonists to $5HT_2$-receptors (ketanserin, ritanserin, and altanserin) and by non-selective serotoninergic antagonist metergoline [11]. Our data can indicate that not only $5HT_{2C}$-receptors are involved in elevation of corticosterone induced by MK-212. In the lowest doses, this agonist modifies behavior, but does not disturb the level of corticosterone in mice. Medium doses of MK-212 increase the content of this hormone, although the highest doses of the agonist produce no effect on the corticosterone level. The increase of plasma corticosterone is mediated not by $5HT_{2C}$-receptors, but by some other pathways.

Thus, low doses of $5HT_{2C}$-receptor agonist MK-212 produce an anxiolytic effect without elevation of plasma corticosterone and without moderation of locomotion in mice.

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TABLE 1. Effect of MK-212 on Mouse Behavior in Open Field Test (M±m)

Index	Intact (n=10)	Physiological saline (n=7)	Dose of MK-212, mg/kg			
			0.1 (n=6)	0.2 (n=7)	0.5 (n=7)	1 (n=8)
Number of crossed squares	45±15	44±11	60±13	49±17	8±2*++	10±6*++
Number of entries to the center	2.4±2.0	0.9±0.6	1.7±0.9	1.9±1.5	0.4±0.4	1.1±1.1
Time spent in the center, sec	3.9±2.5	3.7±2.2	4.3±2.8	4.4±4.1	0.4±0.4	1.1±1.1
Number of rearings	4.9±1.4	2.4±0.6	7.7±2.2+	2.6±1.9	1.4±0.9*	0.9±0.4*+

Note. *p<0.05 compared to the intact mice. ^{+}p<0.05, ^{++}p<0.01 compared to the control mice injected with physiological saline.

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