Blockade of 5-HT$_{2A/2C}$-Type Receptors Impairs Learning in Female Rats in the Course of Estrous Cycle

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Were studied the effects of chronic administration (14 days) of agonist of 5-HT$_{2B/2C}$ serotonin receptors m-CPP (0.5 mg/kg subcutaneously) and agonist of 5-HT$_{2A/2C}$ serotonin receptors ketanserin (0.1 mg/kg intraperitoneally) on conditioned reactions in female rats in different phases of the estrous cycle. Passive avoidance (PA) paradigm and Morris water maze were used as behavioral tests. Chronic administration of m-CPP did not affect PA retrieval during the proestrus and estrus phases, but improved the dynamics of spatial learning in Morris water maze in comparison with control rats. Chronic administration of ketanserin uniformly impaired processes of spatial and nonspatial learning in female rats irrespective to the phase of the estrous cycle. A modulating role of 5-HT$_{2A/2C}$ and 5-HT$_{2B/2C}$ serotonin receptors in process of learning in female rats during the key phases of the estrous cycle was demonstrated.

Key Words: *m-CPP; ketanserin; 5-HT$_2$ serotonin receptors; learning; estrous cycle*

There is a close cooperation between the hypothalamic-pituitary-ovarian hormonal system and the serotonergic neurotransmitter system in the mechanisms of realization of brain cognitive functions [5,6,10,13]. For instance, it was clearly demonstrated that estradiol and serotonin (5-HT) are involved into the mechanisms of learning and memory, sexual behavior, sleep, affective disorders, depression, and schizophrenia [13,14]. 5-HT$_{1A}$- and 5-HT$_{2A/2C}$ subtypes of serotonin receptors are believed to mediate a number of effects of estrogens on the serotonergic system [6,9,14]. Some of serotonergic agents administered 30 min before performance of behavioral tests were shown to modulate the cognitive-affective status in females during various phases of the estrous cycle [7,8]. However, the data are scanty and contradictory due to the lack of comparative analysis of the effects of both agonists and antagonists of different 5-HT receptors on behavioral state of females. Moreover, the interaction between 5-HT and estrogens was primarily studied on ovariectomized females [4,11,12].

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The objective of this study was to investigate role of 5-HT$_{2A/2C}$ and 5-HT$_{2B/2C}$ subtypes of serotonin receptors in conditioned activity in female rats under conditions of natural cyclic changes of their level during the key phases of the estrous cycle.

MATERIALS AND METHODS

The study was carried out on 120 mature albino female Wistar rats weighing 180-200 g and obtained from Rappolovo nursery. The animals were kept under natural illumination and conditions of maximal standardization of temperature and food regimens with free access to water and food. All experiments were performed in the first half of the day (from 9 to 12 a.m.).

All used chemicals were purchased from Sigma. m-CPP was used as agonist of 5-HT$_{2B/2C}$ serotonin receptors (0.5 mg/kg subcutaneously), ketanserin (0.1 mg/kg intraperitoneally) was used as antagonist of 5-HT$_{2A/2C}$ serotonin receptors. The chemicals were dissolved in distilled water with DMSO and administered in a volume of 0.1 ml per rat weighing 200 g. Control rats received the same volume of the vehicle. The

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chemicals were administered for 14 days before the start of behavioral tests.

For each test, the rats were divided into groups of 8-10 animals: rats in the diestrus phase receiving physiological solution (group 1, control), rats in the estrus phase receiving with physiological solution (group 2, control), rats in the proestrus phase receiving physiological solution (group 3, control), rats in the diestrus phase receiving m-CPP (group 4), rats in the estrus phase receiving m-CPP (group 5), rats in the proestrus phase receiving m-CPP (group 6), rats in the diestrus phase receiving ketanserin (group 7), rats in the estrus phase receiving ketanserin (group 8), and rats in the proestrus phase receiving ketanserin (group 9),.

Vaginal smears were obtained daily for 8 days to determine the phase of the estrous cycle (diestrus, proestrus, estrus) using characteristic morphological signs as described previously [2]. Rats with stable 4-day estrous cycle were taken into the experiment.

The rats were trained in the PA paradigm (nonspatial learning) [1] and in Morris water maze (spatial learning) [3].

The data were processed statistically by two-way ANOVA with subsequent Newman–Keuls post-hoc test using software package SPSS 9.0. The differences were statistically significant at $p<0.05$.

RESULTS

Two-way ANOVA revealed significant effects of the test chemical [$F(1,90)=4.27$, $p<0.05$] and hormonal factor (phase of the estrous cycle) [$F(3,90)=3.40$, $p<0.05$], as well as the interaction between them [$F(3,90)=5.81$, $p<0.001$] on PA retrieval in rats (Fig. 1). Post-hoc analysis also revealed significant differences between the control and experimental groups by the results of PA test ($p<0.05$).

It should be noted, that in control rats the retrieval of this reflex was observed only in the phase of diestrus, while during the estrus and proestrus amnesia of PA was observed.

In rats treated with ketanserin (blockade of 5-$HT_{2A/2C}$ serotonin receptors), PA retrieval was impaired irrespective to the phase of the estrous cycle (Fig. 1). Stimulation of 5-$HT_{2B/2C}$ serotonin receptors during estrus and proestrus did to affect PA, *i.e.* parameters of spatial learning in rats treated with m-CPP during the estrous cycle did not differ from those in control rats (Fig. 1).

Two-way ANOVA of the data obtained in Morris water maze during training and test sessions revealed significant effects of the agent ([$F(1,90)=8.32$, $p<0.05$], [$F(3,90)=5.40$, $p<0.05$]) and hormonal factor (phase of estrous cycle) ([$F(3,90)=2.40$, $p<0.05$], [$F(3,90)=2.45$, $p<0.05$]), as well as the interaction between these factors ([$F(3,90)=5.36$, $p<0.001$], [$F(3,90)=2.85$, $p<0.05$]). Post-hoc analysis demonstrated significant differences between the control and experimental groups during the analysis of results obtained in training and testing trials in Morris water maze ($p<0.05$).

In the control group, facilitation of spatial learning was observed during estrus and deterioration during proestrus.

Experimental results suggest that blockade of 5-$HT_{2A/2C}$ serotonin receptors impairs spatial learning irrespectively of the phase of the estrous cycle (Fig. 2). Chronic administration of m-CPP (stimulation of 5-$HT_{2B/2C}$ serotonin receptors) improved spatial learning during the key phases of the estrous cycle (Fig. 2).

Our experiments demonstrated ambiguous effects of selective agonist or antagonist of different subtypes of type 2 serotonin receptors on spatial and nonspatial learning under conditions of natural cyclic changes in estrogen level in the body. In addition, these results proved that the agents affecting serotonergic neurotransmission can modulate cognitive processes in rats in key phases of estrous cycle.

Experiments on PA model showed that chronic administration of 5-$HT_{2A/2C}$ serotonin receptor antagonist ketanserin to rats impaired PA retrieval not only during proestrus and, estrus, but also during diestrus. This suggests that 5-$HT_{2A/2C}$ serotonin receptor blockade levels the differences in nonspatial learning capacity in the course of estrous cycle. Moreover, chronic administration of 5-$HT_{2B/2C}$ serotonin receptor agonist m-CPP to rats did not affect the pattern of spatial learning in investigated phases of estrous cycle.

Model of spatial learning in the Morris water maze revealed similar suppressing effect of ketanserin on

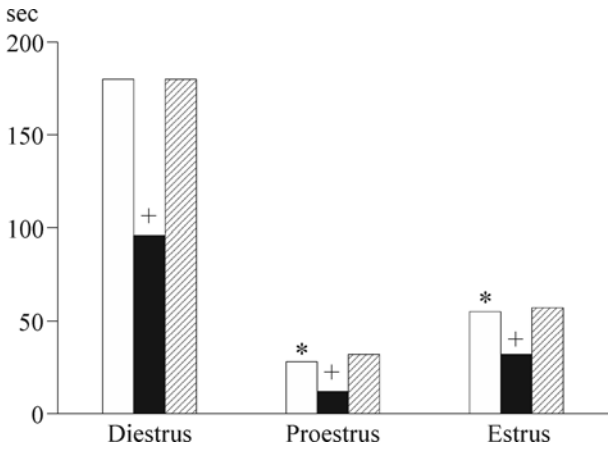

Fig. 1. Effects of chronic administration of ketanserin and m-CPP on PA conditioning and retrieval in female rats. Ordinate: latency of entry into the dark compartment after 24 h. Here and in Fig.2: light bars: control, dark bars: ketanserin, shaded bars: m-CPP. $p<0.05$ in comparison with: *rats in diestrus phase, +control rats in the corresponding phase of the estrous cycle.

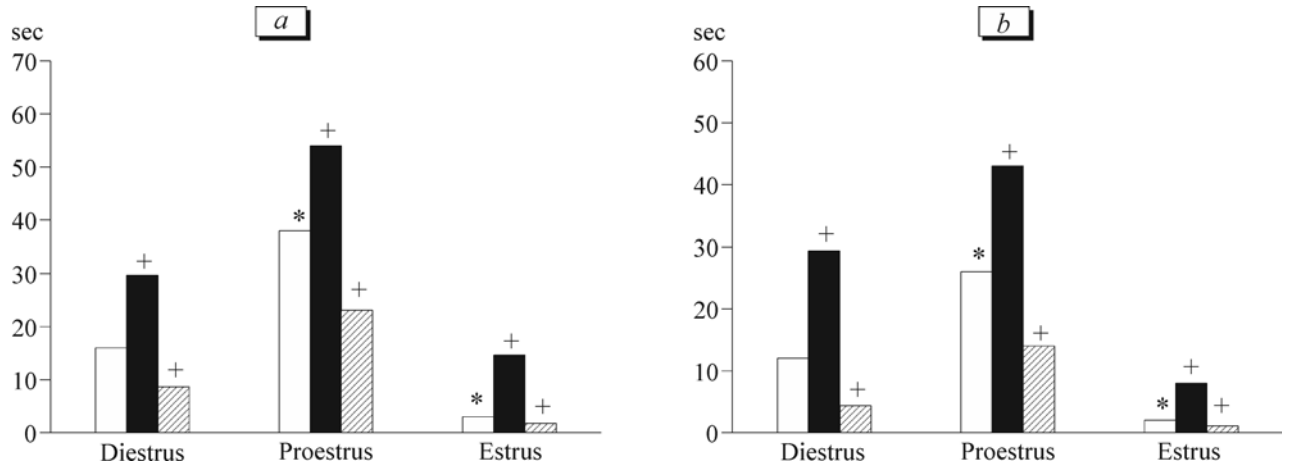

Fig. 2. Effects of chronic treatment with ketanserin and m-CPP on spatial learning of female rats in Morris water maze on day 8. *a*) training session, *b*) test session. Ordinate: time of finding the platform.

learning capacity irrespective of the phase of the estrous cycle. Chronic m-CPP administration improved parameters of spatial learning during the key phases of the estrous cycle in rats, *i.e.* stimulation of central 5-$HT_{2B/2C}$ serotonin receptors also eliminated differences in spatial learning associated with natural hormonal state of the rat. However, it should be noted that the effects of stimulation and blockade of 5-$HT_{2A, 2B, 2C}$ subtypes of serotonin receptors on this type of learning are opposite, but it is not determined by the phase of the estrous cycle.

Opposite effects of these serotonergic compounds on conditioned activity in rats are probably associated with changes in 5-HT metabolism and pattern of the expression of 5-$HT_{2A, 2B, 2C}$ subtypes of serotonin receptors, as well as with changed expression of β-form of estrogen receptors and their binding capacity in brain structures, which are directly relevant to cognitive functions [4-6].

Thus, our experiments demonstrated substantial role of 5-$HT_{2A/2C}$ and 5-$HT_{2B/2C}$ serotonin receptors in cognitive processes at different phases of the estrous cycle in rats and attest to the necessity of further investigation of the effects of serotonergic compounds as agents of pharmacological correction of impaired higher functions of the brain in the course of the estrous cycle.

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