# Effects of Semax against the Background of Dopaminergic Receptor Blockade with Haloperidol

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We studied the neurotropic effects of ACTH(4-10) analog semax against the background of dopaminergic receptors blockade with haloperidol. Intranasal administration of semax (0.05, 0.2, and 0.6 mg/kg) produced virtually no effect on disturbances of orientation and exploratory reactions and motor activity caused by intraperitoneal injection of 0.2 mg/kg haloperidol. By contrast, preliminary administration of 0.05 mg/kg semax prevented haloperidol-induced disturbances in active avoidance conditioning.

**Key Words:** *melanocortins; semax; dopaminergic system; haloperidol*

Melanocortins (ACTH/MSH-like peptides) demonstrate a wide spectrum of physiological effects [9]. These peptides modulate learning and memory, development and regeneration of the nervous system, nociception, inflammatory processes, feeding behavior, and body weight control. The melanocortins accelerate functional recovery of the organism after damage to CNS caused by surgery or drug treatment [10]. A disadvantage of natural melanocortins is short duration of their action, which makes difficult their use in clinical practice. The long-term attempts resulted in the development of heptapeptide semax (Met-Glu-His-Phe-Pro-Gly-Pro), an analog of fragment 4-10 of ACTH. The effects of semax are prolonged compared to those of natural melanocortins [6]. This preparation is used as nootropic and neuroprotective drug [1]. The effects of semax are further studied to assess the spectrum of its physiological activity and to clarify the mechanism of its action.

Semax produces a neuroprotective effect during neurotoxic damage to the dopaminergic system [5]. This effect can result from the modulating effect of semax and stimulation of neurotransmitter release or can be determined by the neurotrophic effect of semax, because it increases the content of neurotrophic factors in CNS [2,8].

There we studied the modulating effect of semax on the cerebral dopaminergic system. To this end, the effects of semax were examined in animals treated with haloperidol, a dopaminergic receptor blocker. We also assessed the nootropic effect of semax against the background of haloperidol treatment impairing cognitive functions in patients due to its side action [11].

## MATERIALS AND METHODS

The study used heptapeptide semax synthesized at the Institute of Molecular Genetics and haloperidol produced by Richter Gedeon. Semax (0.1 ml/kg) was administered via intranasal route in doses of 0.05, 0.2, and 0.6 mg/kg. Haloperidol (1 ml/kg) was injected intraperitoneally in a dose of 0.2 mg/kg 20 min before the start of the experiment.

Experiments were carried out on random-bred albino rats randomized into 4 groups (18-20 animals per group). The rats of experimental groups 1, 2, and 3 rats were treated as follows: *1*) intra-

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peritoneal distilled water and intranasal semax, *2*) intraperitoneal haloperidol and intranasal distilled water, and *3*) intraperitoneal haloperidol and intranasal semax. Controls were treated with equivalent volumes of intraperitoneal and intranasal distilled water.

Motor activity and orientation and exploratory response (OER) were assesses in the open field (OF) test. The rats were placed into the center of OF (round arena 80 cm in diameter) for 2 min. Under red light illumination and in the silence, the latency of exit from the center, the length of run, the number of rearings, and the number of visits to the center were assessed visually.

Learning capacity was assessed using conditioned active avoidance response to noxious stimulation. The conditioned and unconditioned stimuli were bell ring and voltage pulse, respectively. The pulse amplitude was chosen individually for each animal in the range of 70-100 V, while pulse duration of 1 msec and repetition rate of 200 Hz were the same for all rats. The duration of noxious electrical stimulation did not exceed 20 sec. Every a day, each animal received 15 combinations of conditioned and unconditioned stimuli. The drugs were administered only prior to the first learning session: semax (0.05 mg/kg) and haloperidol (0.2 mg/kg) were administered 30 and 20 min, respectively, before the start of learning on experimental day 1. The following parameters were recorded: *1*) the number of conditioned avoidance responses (the number of jumps to a shelf in response to the conditioning stimulus); *2*) the number of escape failures (the rats did not jump to the shelf over 20 sec); and *3*) the number of intersignal reactions (jumps to the shelf before presentation of the conditioning stimulus).

The data were presented as *M*±*SEM* and analyzed statistically using parametric (ANOVA) and

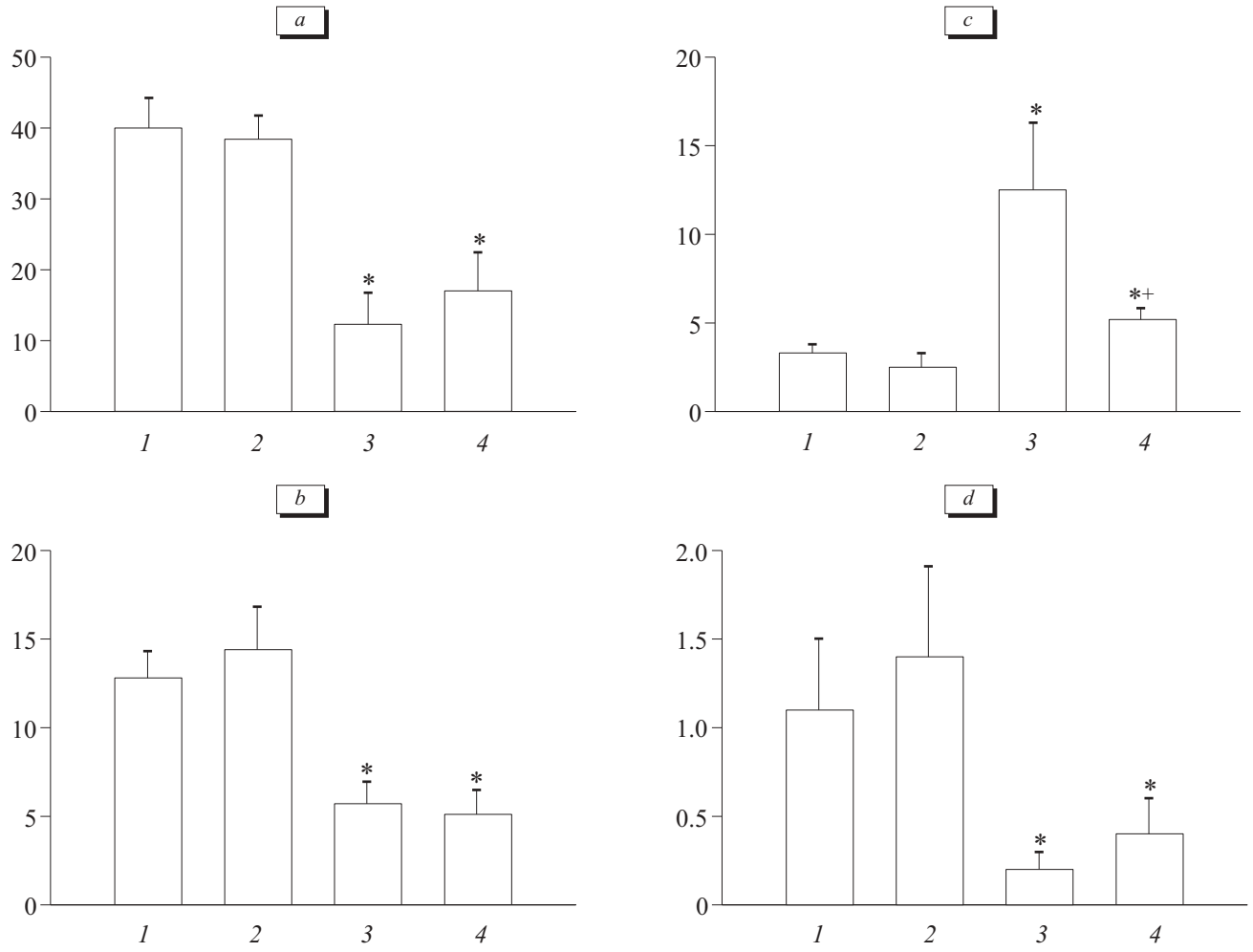


**Fig. 1**. Effects of semax (0.2 mg/kg 30 min before testing), haloperidol (0.2 mg/kg 20 min before testing) and their combined administration on open field behavior in rats. Ordinate: *a*) length of runs (rel. units); *b*) number of rearings; *c*) latency of exit from the center; *d*) number of visits to the center. Here and in Figs 2, 3: *1*) control; *2*) semax; *3*) haloperidol; *4*) semax+haloperidol. $p<0.05$ compared to *control and +haloperidol.

nonparametric (Wilcoxon—Mann—Whitney, and Fisher) tests.

## RESULTS

Semax produced no significant changes in OF behavior (Fig. 1, 2). Injection of haloperidol (0.2 mg/kg) markedly inhibited OER compared to the control. The following indices decreased significantly: the length of runs and the number of rearings and visits to OF. The latency of exit from the center also increased.

Semax injected in doses 0.05 or 0.2 mg/kg 10 min before haloperidol did not modulate the effect of this blocker. Similar to individual administration of haloperidol, we observed inhibition of OER in OF test. It manifested in a significant decrease in the length of run, the number of rearings and visits to OF. The latency of exit from the center also increased. In rats receiving 0.2 mg/kg semax, the increase in the latency of exit from the center was less pronounced than in rats treated with haloperidol alone (Fig. 1). Therefore, when administered 10 min before haloperidol, semax (0.2 mg/kg) moderated its effects on motor activity, but the effects of this dose of semax were minor.

In the next experimental series, a higher dose of semax (0.6 mg/kg) was injected 60 min before OF testing (or 40 min before haloperidol). This dose and protocol were chosen from literature: 1 h after injection of 0.6 mg/kg semax, the neurochemical and behavioral effects of d-amphetamine were potentiated (release of dopamine and motor activity increased compared to individual effects of d-amphetamine) [4]. In our experiments, rats receiving semax and haloperidol and animals treated with haloperidol alone demonstrated significant decrease in the length of runs and number of rearings and visits to OF. The latency of exit from the center also increased. However, the latency was more prolonged after combined administration of drugs than after individual injection of haloperidol: the effects of the blocker were more pronounced (Fig. 2).

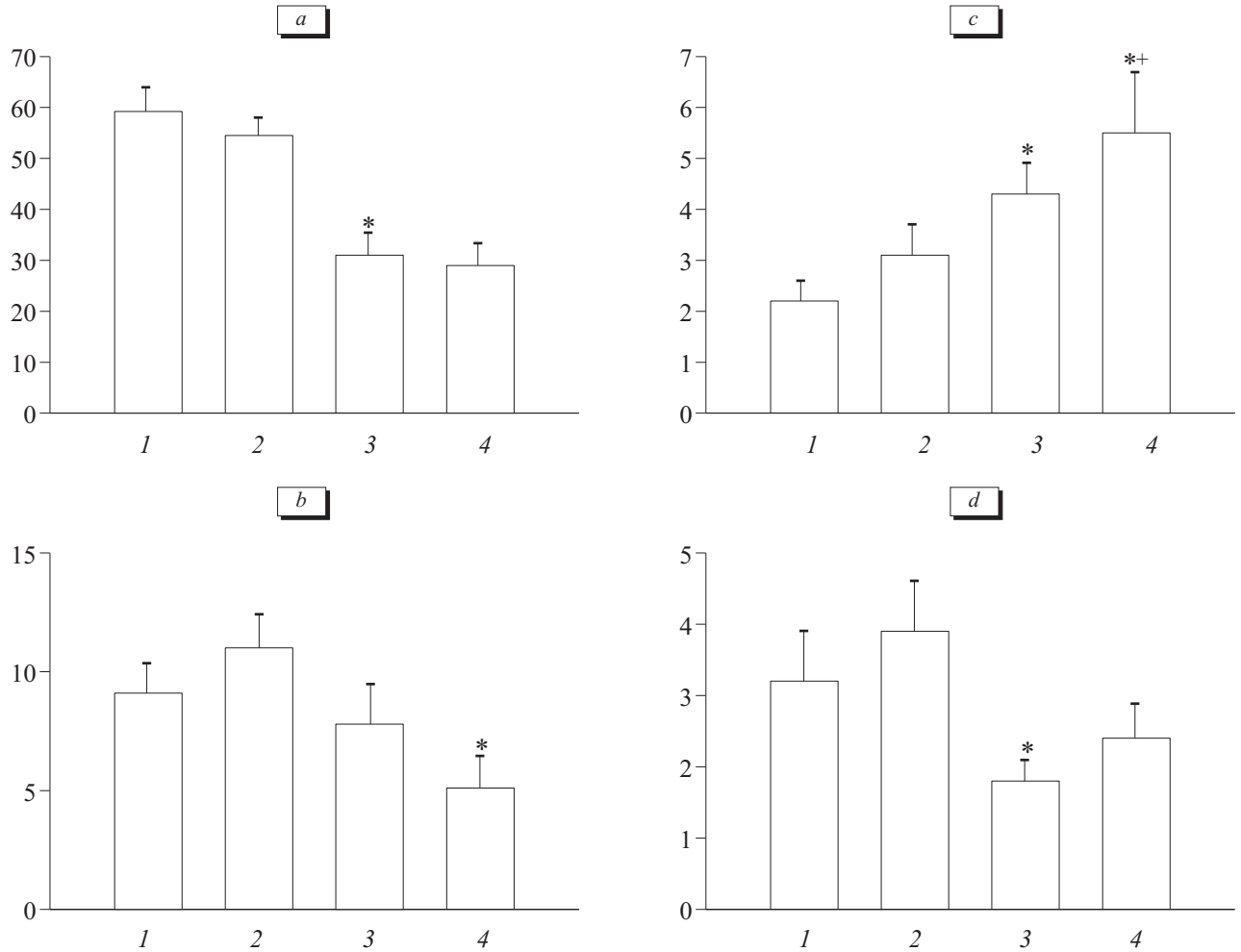


**Fig. 2**. Effects of semax (0.6 mg/kg 60 min before testing), haloperidol (0.2 mg/kg 20 min before testing) and their combined administration on open field behavior in rats.

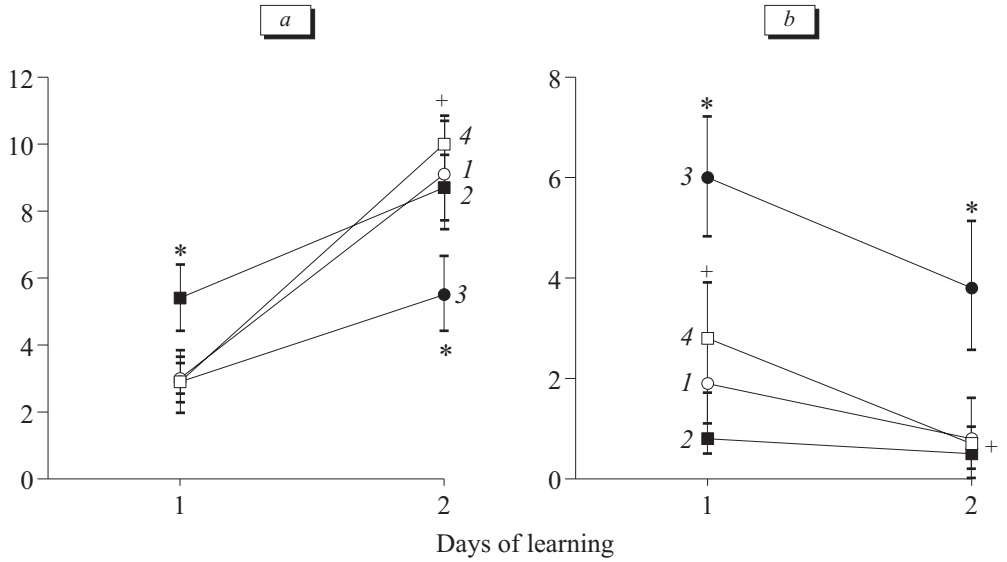


**Fig. 3**. Effects of semax, haloperidol and their combination on parameters of learning conditioned active avoidance. *a*) number of correct reactions; *b*) number of failures.

Thus, depending on the dose semax can reduce or potentiate the effects of haloperidol on motor and explorative activity of rats. However, in both cases the effects of semax were minor.

Testing of conditioned active avoidance response to noxious stimulation showed that semax significantly improved performance on the first day and decreased the number of intersignal reactions on learning day 2 compared to the control (Fig. 3). On the day 1, haloperidol significantly increased the number of escape failures and significantly decreased the number of intersignal reactions in comparison with the control. On learning day 2, rats treated with haloperidol demonstrated significantly lower number of correct responses and significantly higher number of failures in comparison with the corresponding data in the control group. Combined treatment did not modulate learning, but during day 1 the number of failures was lower and the number of intersignal reactions was higher than in rats receiving haloperidol alone; on day 2 the number of correct responses increased and the number of failures decreased compared to haloperidol-treated rats. Thus, preliminary administration of semax in a dose of 0.05 mg/kg prevented conditioned active avoidance disturbances caused by haloperidol.

ACTH-like peptides modulate the cerebral dopaminergic system. They elevate dopamine content in some cerebral structures, stimulate synthesis and potentiate the release of this neurotransmitter in response to external stimulation [7]. Administration of semax in doses of 0.15 and 0.6 mg/kg accelerated metabolism of serotonin, but had no effect on the cerebral dopaminergic system [3]. It was found that injection of semax in a dose of 0.6 mg/kg potentiates behavioral and neurochemical effects of d-amphetamine [4]. Our experiments showed that semax (0.05 mg/kg) prevents conditioned active avoidance disturbances caused by haloperidol. Semax administered in doses of 0.05-0.6 mg/kg had practically no effect on haloperidol-induced moderation of motor activity and OER. Therefore, semax can eliminate adverse side-effects of haloperidol related to learning impairment.

It was previously established that intranasal semax (0.2 mg/kg) increased motor and exploratory activity in animals with neurotoxin-induced damage to the cerebral dopaminergic system. In this case, lower dose of semax (0.05 mg/kg) was ineffective [5]. It can be hypothesized that the normalizing effects of semax in animals with haloperidol- or neurotoxin-induced damage to the cerebral dopaminergic system are mediated by different mechanisms. The effects of semax in neurotoxin-treated animals can result from its neuroprotective action [2,8], which moderates damages and restores the function of reversibly damaged dopamine-producing neurons. By contrast, the normalizing action of semax on acquisition of the conditioned reflex, which is disturbed by haloperidol, can be explained by the compensatory influences of other neurotransmitter systems.

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