PHARMACOLOGY AND TOXICOLOGY

Dipeptide Preparation Noopept Prevents Scopolamine-Induced Deficit of Spatial Memory in BALB/c Mice

A. P. Belnik, R. U. Ostrovskaya, and I. I. Poletaeva*

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The effect of original nootropic preparation Noopept on learning and long-term memory was studied with BALB/c mice. Scopolamine (1 mg/kg) impaired long-term memory trace, while Noopept (0.5 mg/kg) had no significant effect. Noopept completely prevented the development of cognitive disorders induced by scopolamine (blockade of muscarinic cholinergic receptors). Our results confirmed the presence of choline-positive effect in dipeptide piracetam analogue Noopept on retrieval of learned skill of finding a submerged platform (spatial memory). We conclude that the effectiveness of this drug should be evaluated in patients with Alzheimer's disease.

Key Words: *water maze; spatial memory; proline-containing peptides; Noopept; scopolamine*

Cognitive deficit is typical of a variety of brain diseases. In the search of effective and safe drugs for the correction of this disorder recent attention was focused on endogenous regulators, including peptides. The use of peptides as neurotropic drugs is limited by their low stability and insufficient permeability of the blood-brain barrier for these compounds. The class of dipeptides is characterized by higer specific bioavailability for the brain [7].

Pharmacological dipeptide drugs mimicking nonpeptide prototypes with specified neurotropic activity were synthesized at the Institute of Pharmacology. The design of peptides improving cognitive function is based on the hypothesis on the role of oligopeptides containing an endogenous pyrrolidine-carbonic amino acid (pyroglutamic acid or proline) as the ligands of pitative recognition sites for piracetam [1,10]. The study of synthetic pyroglutamine-containing dipeptides with various natural amino acids showed that these compounds improve cognitive function in experimental animals [3].

More than 30 new compounds that belong to a group of acyl-proline dipeptides were developed. Much attention was paid to ethyl ester of N-phenylacetyl-L-prolylglycine (GVS-11), which received the name Noopept [4].

This nootropics improves active avoidance learning. The effect of Noopept on learning was also demonstrated in conditioned passive avoidance paradigm under normal conditions and during memory disorders induced by cholinergic or glutamatergic receptor antagonists [13]. Experiments with isolated neurons from edible snail proved the sensitizing effect of Noopept on cholinergic synaptic transmission [5].

Cholinergic dysfunction is an important pathogenetic factor for cognitive disorders of the brain

Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow; *Biological Faculty, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** rita@nicemail.ru. R. U. Ostrovskaya

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during natural aging, Alzheimer's disease, and Down's syndrome [6]. Impairment of spatial memory is an important sign of these neurodegenerative diseases [9].

Here we studied the effect of Noopept on spatial learning and memory in mice with dysfunction of the cholinergic system induced by muscarinic cholinergic receptor antagonist scopolamine.

MATERIALS AND METHODS

Experiments were performed on male BALB/c mice (n=63) characterized by low concentration of acetylcholine in the hippocampus and cerebral cortex and high sensitivity to drugs impairing cholinergic transmission [11]. The animals (20-23 g) were obtained from Stolbovaya nursery (Russian Academy of Medical Sciences) and maintained in plastic cages (30×70×40 cm, 6-8 mice per cage) under natural light/dark conditions.

A simplified Morris water maze test was used to study spatial learning and memory [12].

The modified test [2] was performed using a plastic pool (70×55×60 cm) filled with room-temperature water whitened with milk. A platform (7 cm in diameter) was placed in a corner 1 cm below water surface.

Our previous studies showed that in this modification of Morris test BALB/c mice cannot reach the platform after single training session (5 consecutive presentations). Therefore, in our experiment the mice were pre-trained to move in the pool with visible platform elevated by 1 cm above water surface. This pretraining trial promotes to the formation of the long-term memory trace.

The pool with visible platform was used on day 1 of the study. Each mouse was put to the pool in 8 different start points along the perimeter. If the animal did not find the platform over 60 sec, it was placed on this platform and maintained there for 10 sec. Thirty minutes after pretraining the mice were randomly divided into 4 groups (15-16 mice per group). Group 1 mice (control) received physiological saline. Group 2 mice received subcutaneous injection of scopolamine in a dose of 1 mg/kg. Group 3 mice received intraperitoneal injection of Noopept in a dose of 0.5 mg/kg. Group 4 mice received scopolamine and Noopept in the specified doses.

The test with hidden platform was performed after 24 h. The mouse was placed in a far corner and the latency of finding the platform was determined. If the mouse did not find the platform within 60 sec, it was placed on this platform and maintained there for 10 sec. Each animal was subjected to 5 consecutive presentations at 15-min intervals. The time spent on the platform was recorded in each presentation. Memory trace testing was performed in the maze with hidden platform after 10 days.

Data processing involved one-way ANOVA. The differences between consecutive presentations were estimated by Student's test.

RESULTS

The stage of pretraining with visible platform shortened the time over which BALB/c mice found the hidden platform. Group 1 animals found the hidden platform over a short time (17-18 sec). The latency of finding the platform in this test did not decrease with each subsequent presentation, which suggests the absence of additional training in this test in control mice. Similar results were obtained in study of skill retention after 10 days (Fig. 1).

After 1 and 10 days, the latency of finding the hidden platform in the group of Noopept-treated mice was similar to that in control animals (Fig. 1). Testing for skill retention on days 1 and 10 after treatment (one-way ANOVA) showed that Noopept had little effect on learning.

The mice receiving scopolamine exhibited longer time to find the platform compared to control animals (Fig. 1). Scopolamine produced a potent effect on learning during skill reproduction on day 1 after treatment (p<0.001). The study of memory retention on day 10 after treatment showed that these mice found the platform over a longer time compared to control animals. The differences were observed in all presentations of this test (p<0.001).

During the first presentation (1 day after scopolamine administration), the mice spent more than 40 sec for finding the platform. On day 10 after scopolamine administration the animals found this platform more rapidly. It can be hypothesized that scopolamine in this dose impairs long-term memory trace, but does not decrease the ability to form a short-term memory trace.

Parameters of learning and memory retrieval in mice receiving scopolamine and Noopept did not differ from the control, but significantly exceeded those in scopolamine-treated animals (Fig. 1). Hence, administration of the nootropic drug to BALB/c mice prevents the negative effect of scopolamine on memory trace formation.

Low learning capacity of BALB/c mice in the water maze can be explained by decreased basal level of acetylcholine in the cortex, hippocampus, and nigrostriatal system [11]. Scopolamine administration aggravates the cholinergic deficit and impairs learning ability in water Morris test.

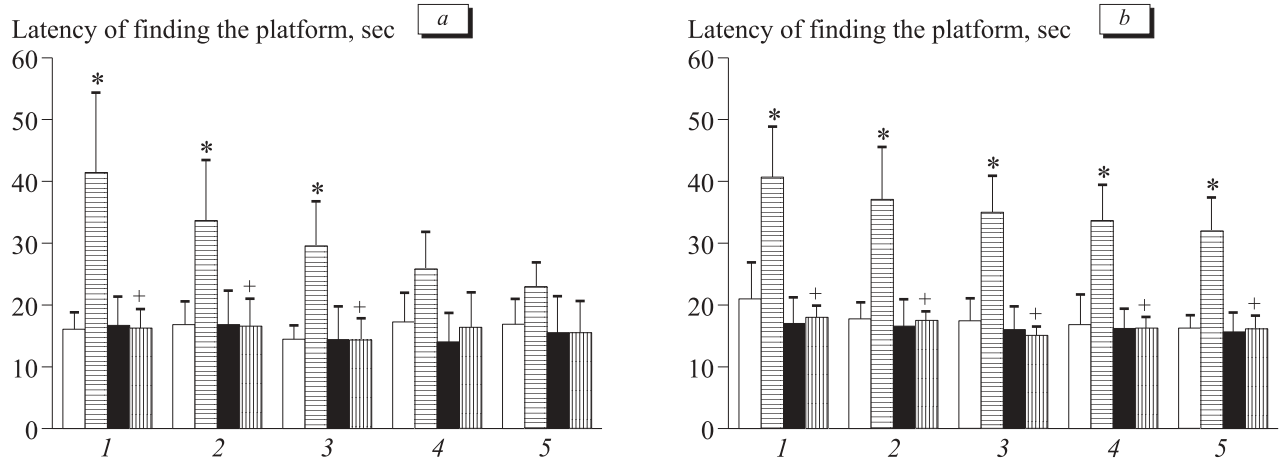

Fig. 1. Effect of scopolamine and Noopept on the latency of finding the hidden platform in simplified Morris water maze test in BALB/c mice. Days 1 (*a*) and 10 after treatment (*b*). *1-5*: presentations in the test. Light bars, control group (physiological saline); horizontal shading, scopolamine (1 mg/kg subcutaneously); dark bars, Noopept (0.5 mg/kg intraperitoneally); vertical shading, Noopept and scopolamine. $p<0.05$: *compared to the control; +compared to the scopolamine group.

Our results indicate that Noopept has no effect on learning to finding the platform in intact mice. However, this drug prevented the development of scopolamine-induced spatial memory deficit. These findings are consistent with published data that nootropic drugs can be effective during cognitive disorders [8].

The ability of Noppept to prevent scopolamine-induced spatial memory deficit not only confirms published data on choline-positive effect of this dipeptide [5], but also suggests that this parameter can be used as a prognostic criterion for the efficiency of this preparation in pathological states associated with deficiency of cholinergic transmission (*e.g.*, Alzheimer's disease). Noopept exhibits high neuroprotective activity [14], has low toxicity, and does not cause side effects [4]. Hence, the effectiveness of Noopept should be evaluated in these patients.

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