Original Nootropic Drug Noopept Prevents Memory Deficit in Rats with Muscarinic and Nicotinic Receptor Blockade

K. S. Radionova, A. P. Belnik, and R. U. Ostrovskaya

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Antiamnesic activity of Noopept was studied on the original three-way model of conditioned passive avoidance response, which allows studying spatial component of memory. Cholinoceptor antagonists of both types (scopolamine and mecamylamine) decreased entry latency and reduced the probability for selection of the safe compartment. Noopept abolished the antiamnesic effect of cholinoceptor antagonists and improved spatial preference.

Key Words: *Noopept; three-way model of conditioned passive avoidance response; cholinergic transmission deficit; spatial memory; antiamnesic properties*

Therapy of neurodegenerative diseases is an urgent problem. This disorder occurs mainly in elderly people [11]. Cholinergic transmission deficit is the major pathogenetic factor for cognitive disorders in natural aging, as well as in Alzheimer's disease and Down syndrome [9]. During the search of new effective and safe pharmaceutics for the correction of cognitive deficit, special attention is paid to endogenous peptide regulators. ACTH analogue HOE427 is effective at initial stages of Alzheimer's disease, which results form choline-positive activity of the compound [14]. The use of peptides in clinical practice is limited by poor permeability of the blood-brain barrier and low enzyme resistance. As differentiated from complex peptides, the class of dipeptides is characterized by relatively high specific bioavailability for the brain [6].

One of the approaches developed at the Institute of Pharmacology suggests the development of dipeptides, which are structurally close to a non-peptide neurotropic compound and active fragment of the peptide with similar neurotropic activity [8]. Several proline-containing dipeptides were synthesized taking into account the peptidergic mechanism of the action of piracetam (parent compound for the class of nootropic drugs). Piracetam and AVP_{4-9} (major metabolite of vasopressin with N-terminal Pyr-Asp) served as prototypes of these dipeptides [9].

Among a variety of proline-containing dipeptides, N-phenylacetyl-L-prolylglycine ethyl ester (GVS-111) exhibits maximum nootropic activity. This compound received the name Noopept [4]. This nootropic drug produces a positive effect on learning in a conditioned active avoidance response and two-way model of a conditioned passive avoidance response (CPAR) [4,12]. Experiments on isolated neurons from edible snail revealed a choline-positive effect of Noopept [3]. Published data show that neurodegenerative diseases are accompanied by impairment of spatial memory [7].

Here we studied the effect of Noopept on learning and spatial memory after treatment with muscarinic receptor antagonist scopolamine and nicotinic receptor antagonist mecamylamine.

V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** rita@nicemail.ru. R. U. Ostrovskaya

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats (n=37) weighing 200-330 g and obtained from the Stolbovaya nursery (Russian Academy of Medical Sciences). The animals were maintained in a vivarium under standard conditions and natural light/dark cycle and had free access to food and water.

Original three-way model was used for CPAR acquisition and performance [2]. As distinct from the two-way model, this approach allows studying the spatial component of memory. The chamber (60×30×35 cm) was divided into 3 similar compartments with electrode floor separated from each other by walls with doors. The central compartment was illuminated. The left and right compartments were dark.

Experiments were performed in 2 series. Series I was conducted on 40 animals (3 groups of 12-15 specimens each). Group 1 animals (passive control) were treated twice with 0.9% solution of NaCl. Group 2 animals (active control) received scopolamine (1.35 mg/kg subcutaneously) and 0.9% solution of NaCl. Scopolamine and Noopept (0.5 mg/kg intraperitoneally) were injected to group 3 animals. Series II was conducted on 33 animals (3 groups of 10-12 specimens each). Group 1 animals (passive control) were treated twice with 0.9% solution of NaCl. Group 2 animals (active control) received mecamylamine (2 mg/kg subcutaneously) and 0.9% solution of NaCl. Mecamylamine and Noopept (0.5 mg/kg intraperitoneally) were injected to group 3 animals.

The study was performed for 3 days. Experimental animals were familiarized with the chamber on day 1. Both doors of the dark compartments were open. Each rat was placed in the start compartment for 3 min. Due to innate hole reflex, the rat moved to the dark compartment (left or right, at random). The latency of entering the dark compartment was recorded.

CPAR was trained on day 2. Scopolamine and mecamylamine were injected to group 2 and 3 animals 30 min before CPAR learning. Group 1 animals received an equivalent volume of 0.9% NaCl. Noopept was injected to treated rats 15 min before CPAR training. Control animals received an equivalent volume of 0.9% NaCl. The animal was placed in the illuminated central compartment. The door of the left compartment was closed. Five inescapable stimuli (electric current 1 mA) were delivered through the electrified floor at 5-sec intervals. We recorded the latency of entering the dark compartment and horizontal locomotor activity of rats.

Testing was performed on day 3. The rats were placed in the start compartment. Both doors were open. The selection of the dark compartment (right dangerous or left safe compartment) was recorded. The latency of entering this compartment was measured. The animals were examined for 5 min. Spatial preference was estimated from the following 2 parameters: number of animals remaining in the central compartment and coefficient of safe compartment preference (C_{SCP}). C_{SCP} was calculated as the ratio of the number of rats preferring safe compartment to the number of animals preferring dangerous compartment. Memory retention was tested on day 14.

The effectiveness of CPAR performance was evaluated from antiamnesic (Aa) activity of the test compound [12]:

$$Aa=\frac{\Delta Latency_{drug}-\Delta Latency_{amnesia})}{\Delta Latency_{control}-\Delta Latency_{amnesia}}\times 100\%,$$

where $\Delta Latency=Latency_{testing}-Latency_{acquisition}$.

Statistical treatment was performed with Biostat software. Differences in the latency of entering the dark compartment were evaluated by nonparametric Mann—Whitney U test. Differences in spatial preference were estimated by Fischer exact test.

RESULTS

No intergroup differences were found in the latency of entering the dark compartment during CPAR training (day 2, 10-15 sec, Figs. 1 and 2). These data reflect homogeneity of experimental samples. In animals of the passive control group, the latency of entering the dark compartment increased after 24 h (p<0.01) and 14 days (p<0.05), which attests to high effectiveness of learning and memory retention in the delayed period.

Series I showed that the latency of entering the dark compartment for scopolamine-treated animals after 24 h is lower compared to specimens of the passive control group (p<0.01). These data confirm the notion on amnesic activity of scopolamine. Similar results were obtained for active control rats in the delayed period (p<0.01, Fig. 1). After 24 h, the latency of entering the dark compartment for treated rats was higher compared to scopolamine-treated animals (Fig. 1). However, these differences were statistically insignificant. Aa was 24%. The antiamnesic effect of Noopept was more pronounced on day 14. The latency of entering the dark compartment for rats receiving Noopept and scopolamine significantly differed from that for animals pretreated with scopolamine (p<0.01). Moreover, this parameter in rats of the Noopept+sco-

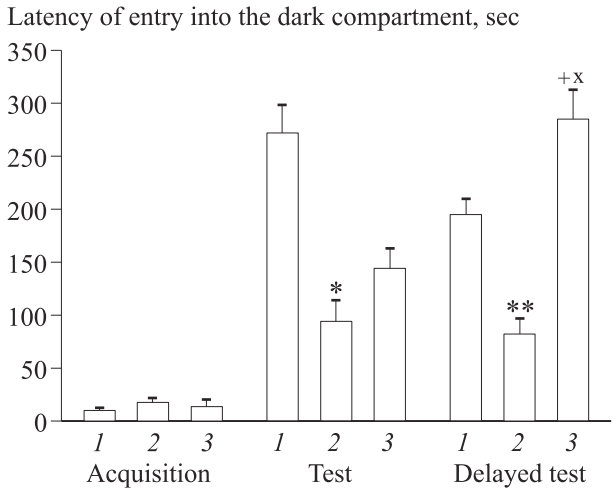

Fig. 1. Effect of Noopept on learning during scopolamine-induced amnesia. Control (*1*); scopolamine, 1.35 mg/kg (*2*); and Noopept (0.5 mg/kg) and scopolamine. **p*<0.01 compared to the control, test after 24 h; ***p*<0.01 compared to the control, delayed test after 14 days; ^{+}p<0.01 compared to scopolamine, delayed test after 14 days; ^{x}p<0.05 compared to the control, delayed test after 14 days.

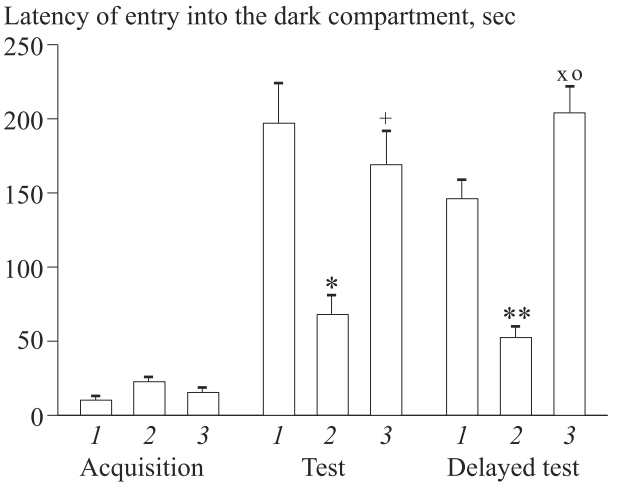

Fig. 2. Effect of Noopept on learning during mecamylamine-induced amnesia. Control (*1*); mecamylamine, 2 mg/kg (*2*); and Noopept (0.5 mg/kg) and mecamylamine. **p*<0.01 compared to the control, test after 24 h; ^{+}p<0.01 compared to mecamylamine, test after 24 h; ***p*<0.05 compared to the control, delayed test after 14 days; ^{x}p<0.01 compared to mecamylamine, delayed test after 14 days; ^{o}p<0.05 compared to the control, delayed test after 14 days.

polamine group was much higher than in animals of the passive control group (p<0.05, Fig. 1). Aa in the delayed period was 189%. These data illustrate high antiamnesic activity of Noopept during the impairment of muscarinic cholinergic transmission. Our findings confirm previous data that Noopept improves long-term memory [12].

Series II showed that the entry latency for mecamylamine-treated rats after 24 h and 14 days is much lower than for animals of the passive control group (p<0.01, Fig. 2). Noopept compensated for the amnesic effect of nicotinic receptor antagonist in rats. Differences in the entry latency between treated rats and mecamylamine-treated animals were significant after 24 h (p<0.01, Aa=73.7%) and, particularly, on day 14 (p<0.01, Aa=151%). Similarly to rats with scopolamine-induced amnesia, the entry latency for animals receiving mecamylamine and Noopept during the delayed period was much higher compared to specimens of the passive control group (p<0.05, Fig. 2).

In both series, the majority of passive control animals remained in the central compartment. Spatial memory deficit was observed in animals receiving one of the cholinoceptor antagonists. We revealed a significant decrease in the number of animals remaining in the central compartment. C_{SCP} decreased under these conditions (Table 1). Scopolamine produced greater impairment of spatial memory than mecamylamine, which is consistent with published data [11]. Both series showed that administration of Noopept is followed by a significant increase in the number of animals remaining in the central compartment (p<0.05, Table 1). Se-

TABLE 1. Spatial Distribution of Animals on the Model of Scopolamine-Induced and Mecamylamine-Induced Amnesia

Series		Selected the dangerous compartment	Remained in the start compartment	Selected the safe compartment	C_{SCP}
I	passive control, n=13	1	9	3	3
	scopolamine, n=12	6	1*	5	0.83
	scopolamine+Noopept, n=15	2	7^{+}	6	2
II	passive control, n=12	2	7	3	1.5
	mecamylamine, n=10	5	1^{o}	4	0.8
	mecamylamine+Noopept, n=11	2	7^{x}	2	1

Note. **p*<0.005 compared to the passive control; ^{+}p<0.05 compared to scopolamine-receiving animals; ^{o}p<0.05 compared to the passive control; ^{x}p<0.05 compared to mecamylamine-receiving animals.

ries I showed that C_{SCP} increases in Noopept-treated rats (Table 1).

The study on the original three-way model of CPAR showed that Noopept has the antiamnesic effect after treatment with muscarinic and nicotinic receptor antagonists (scopolamine and mecamylamine, respectively). Experiments on a modified model of CPAR showed that impairment of spatial memory is more pronounced under conditions of muscarinic receptor blockade. Noopept prevents the development of memory disorders. The effect of this dipeptide preparation on memory is not related to changes in horizontal activity of animals. Both series showed that the average number of crossed squares in rats of all groups is 10-15. These data support the hypothesis on a specific mnemotropic effect of Noopept.

Our results are consistent with published data that Noopept normalizes spatial orientation and memory in the Morris water maze during scopolamine-induced amnesia [1] and olfactory bulbectomy (model of Alzheimer's disease) [13]. Noopept exhibits choline-positive activity and produces a strong nootropic and neuroprotective effect on various models of brain injury [5,13]. Hence, Noopept holds much promise for the therapy of neurodegenerative diseases.

REFERENCES

1. A. P. Belnik, R. U. Ostrovskaya, and I. I. Poletaeva, *Byull. Eksp. Biol. Med.*, **143**, No. 4, 407-410 (2007).
2. A. N. Inozemtsev, A. P. Belnik, and R. U. Ostrovskaya, *Eksper. Klin. Farmakol.*, **70**, No. 3, 67-69 (2007).
3. R. U. Ostrovskaya, T. Kh. Mirzoev, F. A. Firova, *et al*., *Ibid*., **64**, No. 2, 11-14 (2001).
4. R. U. Ostrovskaya, T. A. Gudasheva, T. A. Voronina, and S. B. Seredenin, *Ibid*., **65**, No. 5, 66-72 (2002).
5. K. S. Us, V. A. Kraineva, I. P. Galaeva, *et al*., *Psikhofarmakol. Biol. Narkol*., **6**, Nos. 1-2, 1156-1164 (2006).
6. G. Fricker and J. Drewe, *J. Pept. Sci*., **2**, No. 4, 195-211 (1996).
7. G. Gron, I. Brandenburg, A. P. Wunderlich, and M. W. Riepe, *Neurobiol. Aging*, **27**, No. 1, 78-87 (2006).
8. T. A. Gudasheva, T. A. Voronina, *et al*., *Eur. J. Med. Chem*., **31**, 151-157 (1996).
9. A. D. Lawrence and B. J. Sahakian, *Neurochem. Res*., **23**, No. 5, 787-794 (1998).
10. L. Leblond, C. Beaufort, F. Delerue, and T. P. Durkin, *Behav. Brain Res*., **128**, No. 1, 91-102 (2002).
11. K. Maubach, *Expert Opin. Investig. Drugs*, **12**, No. 9, 1571-1575 (2003).
12. R. U. Ostrovskaya, T. A. Gudasheva, S. S. Trofimov, *et al*., *Biological Basis of Individual Sensitivity to Psychotropic Drugs*, Eds. S. B. Seredenin *et al*., Edinburgh (1994), pp. 79-91.
13. R. U. Ostrovskaya, M. A. Gruden, N. A. Bobkova, *et al*., *J. Psychopharmacol.*, **21**, No. 6, 611-619 (2007).
14. D. S. Reddy, *Methods Find. Exp. Clin. Pharmacol*., **20**, No. 3, 249-277 (1998).