Noopept Stimulates the Expression of NGF and BDNF in Rat Hippocampus

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We studied the effect of original dipeptide preparation Noopept (N-phenylacetyl-L-prolylglycine ethyl ester, GVS-111) with nootropic and neuroprotective properties on the expression of mRNA for neurotropic factors NGF and BDNF in rat hippocampus. Expression of NGF and BDNF mRNA in the cerebral cortex and hippocampus was studied by Northern blot analysis. Taking into account the fact that pharmacological activity of Noopept is realized after both acute and chronic treatment, we studied the effect of single and long-term treatment (28 days) with this drug. Expression of the studied neurotropic factors in the cerebral cortex was below the control after single administration of Noopept, while chronic administration caused a slight increase in BDNF expression. In the hippocampus, expression of mRNA for both neurotrophins increased after acute administration of Noopept. Chronic treatment with Noopept was not followed by the development of tolerance, but even potentiated the neurotrophic effect. These changes probably play a role in neuronal restoration. We showed that the nootropic drug increases expression of neurotrophic factors in the hippocampus. Our results are consistent with the hypothesis that neurotrophin synthesis in the hippocampus determines cognitive function, particularly in consolidation and delayed memory retrieval. Published data show that neurotrophic factor deficiency in the hippocampus is observed not only in advanced Alzheimer's disease, but also at the stage of mild cognitive impairment (predisease state). In light of this our findings suggest that Noopept holds much promise to prevent the development of Alzheimer's disease in patients with mild cognitive impairment. Moreover, therapeutic effectiveness of Noopept should be evaluated at the initial stage of Alzheimer's disease.

Key Words: *Noopept; neurotrophic factors; hippocampus; Alzheimer's disease*

Cognitive deficit is typical of various diseases, including stroke, chronic dyscirculatory encephalopathy, neurodegenerative disorders, brain trauma, neuroinfection, and mental retardation in children. Memory deficit in these diseases is accompanied by structural changes in brain tissue of different severity. Hence, it is important to develop high-efficiency drugs that improve cognitive function and have the neuroprotective effect. The synthesis of endogenous peptide derivatives is of considerable importance. A new method developed at the Institute of Pharmacology suggests the synthesis of dipeptide drugs, which imitate the structure of a nonpeptide substance and active site of the peptide

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with neurotropic properties. N-acyl proline derivatives with nootropic activity were synthesized from the active fragment of the major vasopressin metabolite AVP_{4-9} and nonpeptide prototype of piracetam [8]. Among these preparations, N-phenylacetyl-L-prolylglycine ethyl ester (GVS-111, Noopept) is characterized by high activity, low toxicity, and peroral bioavailability. This drug was subjected to detailed studies. Our previous experiments showed that Noopept normalizes cognitive function [5] and facilitates synaptic transmission in the cerebral cortex [4] and hippocampus [7]. Noopept was highly effective on experimental models of Alzheimer's disease for various types of cholinergic deficit in the brain, including chronic treatment with scopolamine [5], olfactory bulbectomy [11], and administration of β-amyloid into nucleus basalis of Meynert [6]. Electrophysiological studies on isolated neurons of *Helix lucorum* showed that Noopept increases sensitivity of the postsynaptic membrane to acetylcholine [5].

A close relationship exists between the cholinergic system and neurotrophins. The loss of cholinergic neurons in Alzheimer's disease is associated with a deficiency of neurotrophic factors NGF and BDNF and their receptors. Neurotrophins provide synaptic plasticity and stable interaction of neurons. They play an important role in cognitive function. Neurotrophins induce differentiation and growth of neurons and improve reparative processes in the nervous system. Hence, they play the major role in the mechanisms of neuroprotection during brain injury (*e.g.*, neurodegenerative diseases) [9]. *In vivo* studies showed that Noopept produce a strong neuroprotective effect during experimental cerebral ischemia [5]. This effect of Noopept was also revealed in *in vitro* experiments with neuronal cultures of cerebellar granule neurons from rats [1] and cerebral cortical neurons of aborted fetuses with Down syndrome [12]. Another peptide analogue of piracetam and AVP_{4-9}, pGlu Asn NH_2, increases the expression of mRNA for BDNF and NGF in hippocampal tissue culture [3].

Here we studied the effect of Noopept on expression of two major neurotrophic factors, BDNF and NGF. Taking into account the fact that pharmacological activity of Noopept is realized during acute and chronic treatment, we studied the effect of single and long-term treatment with this drug.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 240-300 g and obtained from the Stolbovaya nursery (Russian Academy of Medical Sciences). The animals were housed in plastic cages (30×70×40 cm, 6-8 rats per cage) under natural light/dark cycle and had free access to water and food (MEST combined food). The study was conducted according to the rules of researches with experimental animals (Order of the USSR Ministry of Health, No. 755, 12.08.1977).

TABLE 1. Primers for Study of Neurotrophin Gene Expression

BDNFf	5'-GGTATCCAAAGGCCAACTGA-3'
BDNFr	5'-CTTATGAATCGCCAGCCAAT-3'
NGFf	5'-TGT GCC TCA AGC CAG TGA AAT T-3'
NGFr	5'-TCC ACA GTG ATG TTG CGG GTC T-3'

The animals were divided into 4 groups of 6-8 rats each. Group 1 and 3 rats intraperitoneally received 0.9% NaCl (single injection and 28-day treatment, respectively). Noopept (0.5 mg/kg) was injected intraperitoneally to group 2 (single injection) and 4 rats (28-day treatment). The rats were decapitated 30 min after single treatment or last injection of Noopept. The brain was prepared. The cerebral cortex and hippocampus were isolated and immediately placed in liquid nitrogen.

Total RNA was isolated with TRYzol reagent (Invitrogen). The quality of isolation and RNA concentration were estimated from light absorption at 260 and 280 nm. The study was performed with 2 μg RNA from each sample. RNA was separated in 1.5% denaturing agarose gel with 10% formaldehyde. RNA was transferred to a Hybond-XL membrane (Amersham). DNA probes for hybridization were complementary to NGF and BDNF gene sequences. They were labeled with [α-32P]dATP. The probes were synthesized using NGFf/NGFr and BDNFf/BDNFr primers (Table 1). The membrane was washed and exposed with a Retina roentgen film. Before hybridization, the membranes were stained with methylene blue (0.5% sodium acetate, pH 5.2; and 0.04% methylene blue) for measuring RNA concentration in the sample (control for application). Target mRNA concentration in each sample was equalized by total RNA concentration. The values were normalized by the control (one unit).

RESULTS

Expression of the neurotropic factors in the cerebral cortex slightly decreased after acute administration of Noopept. However, chronic treatment with Noopept for 28 days slightly increased expression of BDNF (Table 2). The expression of mRNA for both neurotrophins in rat hippocampus

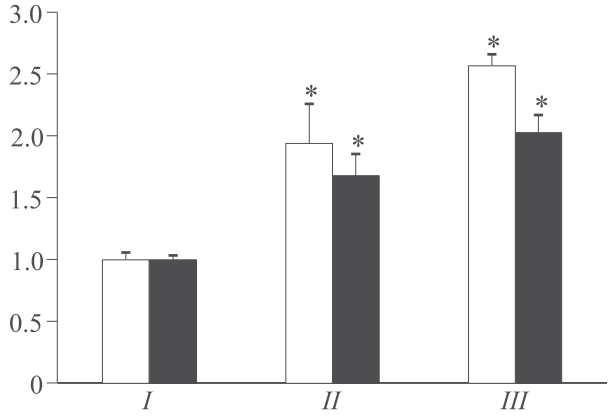

Fig. 1. *In vivo* effect of acute and chronic treatment with Noopept on mRNA expression for NGF and BDNF in rat hippocampus. (*I*) Groups 1 and 3; (*II*) group 2; (*III*) group 4. Light bars, NGF mRNA; dark bars, BDNF mRNA. Abscissa: mRNA concentration, relative to the control (one unit).

increased after acute administration of Noopept. Chronic treatment with Noopept was followed by a greater increase in neurotrophin mRNA expression in the hippocampus. It should be emphasized that the expression of NGF mRNA increased more significantly than that of BDNF mRNA (Fig. 1). Our results indicate that chronic administration of Noopept is not accompanied by the development of tolerance, but potentiates the neurotrophic effect. Hence, long-term treatment with Noopept can improve neuronal recovery.

We found that the nootropic drug increases gene expression for neurotrophic factors in the hippocampus. Our results are consistent with the hypothesis that neurotrophin synthesis in the hippocampus determines long-term hippocampal potentiation (electrophysiological equivalent of memory) [10]. Published data show that neurotrophic factor deficiency in the hippocampus plays the major role in the development of cognitive deficit. The signs of hippocampal hypotrophy are observed not only in Alzheimer's disease, but also at the stage of mild cognitive impairment (pre-disease state) [13]. It is important that Noopept exhibits activity in several types of hippocampus-dependent behavior. For example, Noopept improves the contextual component of a conditioned freezing response. It should be noted that administration of Noopept 24 h after learning has the same effect [5]. In the water maze task (another type of hippocampus-dependent behavior), administration of Noopept before learning improved memory retrieval under conditions of spatial memory deficit due to cholinergic receptor blockade [2]. This effect was observed in the early and delayed period (24 h and 10 days after learning, respectively). The state of amnesia due to administration of a muscarinic receptor antagonist scopolamine or nicotinic receptor antagonist mecamylamine was studied in the spatial three-choice test of conditioned passive avoidance response. The antiamnesic effect of Noopept did not disappear, but even increased on day 10 after treatment. Previous studies showed that BDNF and NGF play a role in the transition from consolidation to long-term memory and delayed memory retrieval [14]. These data suggest that the delayed effects of Noopept on memory are related to neurotrophic activity.

The ability of Noopept to increase neurotrophic factor expression plays a role not only in cognitive activity, but also in a variety of neuroprotective properties of this dipeptide (*e.g.*, during ischemic stroke and brain trauma). Long-term administration of Noopept is accompanied by an increase in the neurotrophic effect. Hence, Noopept holds promise for the reparative phase after brain trauma. Our findings extend the range of clinical applications for Noopept. Previous studies showed that Noopept increases the production of antibodies against β-amyloid [11], has a cholinosensitizing effect, and protects neurons from lipid peroxidation products and excessive amounts of calcium, glutamate, and proinflammatory cytokines [5]. We showed that Noopept has neurotrophic properties. Our results indicate that Noopept holds much promise to prevent the development of Alzheimer's disease in patients with mild cognitive impairment. Moreover, the therapeutic effectiveness of Noopept should be evaluated at the initial stage of Alzheimer's disease. Low toxicity of

TABLE 2. Effect of Acute and Chronic Treatment with Noopept on mRNA Expression for BDNF and NGF (*M*±*SEM*)

Region	0.9% NaCl, acute administration		Noopept (0.5 mg/kg), acute administration		0.9% NaCl, administration for 28 days		Noopept (0.5 mg/kg), administration for 28 days	
	BDNF	NGF	BDNF	NGF	BDNF	NGF	BDNF	NGF
Cerebral cortex	1.00±0.03	1.00±0.04	0.60±0.11	0.71±0.15	1.00±0.03	1.00±0.04	1.26±0.03*	1.02±0.04
Hippocampus	1.00±0.03	1.00±0.06	1.68±0.17*	1.94±0.32*	1.00±0.03	1.00±0.06	2.03±0.14*	2.57±0.10*

Note. *$p<0.05$ compared to the control.

Noopept is associated with the endogenous nature of its major metabolites. That is why Noopept can be used for long-term preventive therapy.

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