Noopept Efficiency in Experimental Alzheimer Disease (Cognitive Deficiency Caused by β-Amyloid$_{25-35}$ Injection into Meynert Basal Nuclei of Rats)

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Experiments on adult Wistar rats showed that injection of β-amyloid$_{(25-35)}$ (2 μg) into Meynert basal nuclei caused long-term memory deficiency which was detected 24 days after this injection by the memory trace retrieval in conditioned passive avoidance reflex (CPAR). The effects of noopept, an original nootropic and neuroprotective dipeptide, on the severity of this cognitive deficiency were studied. Preventive (for 7 days before the injury) intraperitoneal injections of noopept in a dose of 0.5 mg/kg completely prevented mnestic disorders under conditions of this model. Noopept exhibited a significant normalizing effect, if the treatment was started 15 days after the injury, when neurodegenerative changes in the basal nuclei, cortex, and hippocampus were still acutely pronounced. The mechanisms of this effect of the drug are studied, including, in addition to the choline-positive effect, its multicomponent neuroprotective effect and stimulation of production of antibodies to β-amyloid$_{(25-35)}$. Noopept efficiency in many models of Alzheimer disease, its high bioavailability and low toxicity suggest this dipeptide for further studies as a potential agent for the treatment of this condition (initial and moderate phases).

Key Words: *noopept; Alzheimer disease model; Meynert giant cell nucleus; β-amyloid$_{(25-35)}$*

Alzheimer disease (AD) is a chronic neurodegenerative disease leading to progressive deterioration of cognitive functions. The disease was for the first time described more than 100 years ago (in 1906), but casual therapy of this severe disabling disease was considered impossible until recent time. The results of recent basic studies of pathogenetic mechanisms of AD suggest the possibility of development of new therapeutic methods modulating the mechanisms triggering AD. These studies imply the development of models simulating the important pathogenetic mechanisms of this disease. The main component of senile plaques in AD is amyloid$_{(1-42)}$. Its neurotoxic effects are reproduced by hydrophobic C-terminal fragment (β-amyloid$_{(25-35)}$; Aβ$_{(25-35)}$) [11]. It was shown that injection of Aβ$_{(25-35)}$ into Meynert basal giant cell nuclei causes disseminated degenerative changes in the neurons of the frontal cortex and hippocampus, manifesting by different forms of cognitive deficiency [8,10]. Suppression of long-term hippocampal potentiation, induced by Aβ$_{(25-35)}$), is the electrophysiological equivalent of these disorders [7].

An original concept of creation of psychotropic agents was developed during several years at Institute of Pharmacology by T. A. Gudasheva: di-

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peptides simulating the structure of non-peptide neurotropic preparations and peptide active center with predominating activity of the corresponding type are created [9]. Comparison of the structure of standard nootrope (piracetam) with the structure of N-terminal fragment of the main fragment of vasopressin ("memory peptide") led to creation of a series of acylproline-containing dipeptides [14], from which N-phenylacetyl-L-prolylglycine ethyl ether (GVC-111; Noopept) was selected. Detailed studies detected a complex of nootropic and neuroprotective characteristics in noopept [2], manifesting, on AD models, for example, under conditions of chronic blockade of cholinergic receptors [3] and olfactory bulbectomy [12].

We studied noopept effect on the model of memory disorders induced by $A\beta_{(25-35)}$ injection in Meynert basal nuclei.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (280-300 g) kept under standard vivarium conditions at natural light and free access to water and food. The animals were randomly divided into 4 groups (Fig. 1). Group 1 (passive control, n=9) consisted of animals injected with 0.9% NaCl into the left and right Meynert nuclei and after 15-day interval receiving daily intraperitoneal (i/p) injections of saline for 7 days; after this treatment the rats were trained to perform conditioned passive avoidance reflex (CPAR), and after 24 h the retention of memory trace was tested. Group 2 animals (active control, n=10) were injected with $A\beta_{(25-35)}$ in Meynert nuclei; subsequent stages were the same as in group 1. In group 3 (noopept therapy, n=9) the rats were also injected with $A\beta_{(25-35)}$ in Meynert nuclei and after a 15-day interval were daily injected with noopept (0.5 mg/kg i/p; 0.2 ml/100 g) during 7 days; training and testing were carried out as in groups 1 and 2. Group 4 (preventive noopept therapy, n=9) animals were injected with noopept in the same dose during 7 days and after a 7-day interval were injected with amyloid in Meynert nuclei; training was carried out after 15-day interval, and testing after 24 h. Bilateral injections of $A\beta_{(25-35)}$ in Meynert nuclei in a dose of 2 μg, diluted in 1 μl saline, to animals of groups 2, 3, and 4 or the same volume of saline to group 1 animals were carried out on narcotized rats (pentobarbital 50 mg/kg, i/p) placed in a stereotaxic apparatus; Meynert nucleus coordinates: AP, -1.4; OL, 2.7; H, 8.7.

Passive avoidance was trained in a box consisting of 2 compartments (lighted by a 40-W lamp and dark), 25×40×25 cm each, with a floor from metal rods. The compartments were connected with a 8×8 cm hole in the wall with a guillotine door. For CPAR training the animal was placed into the light compartment with its tail to the closed hole. After 120 sec the guillotine door was opened and the animal ran into the dark compartment, due to congenital hole reflex intrinsic of rodents; the time of this passage was recorded (LP_1; manifestation of the hole reflex). Immediately after all 4 paws of the rat were in the dark compartment, the door between the compartments was closed and the rat received electrocutaneous stimulation (0.8 mA, 3 sec) through the floor rods, after which it was immediately removed from box and placed into its home cage. The trained habit was tested after 24 h. The rat was placed into the light compartment with the door open and the time of passage to the dark compartment was recorded (LP_2). The maximum duration of observation was 180 sec (from the moment of door opening on the day of training and from the moment of placing the rat into the box on the day of testing).

The results were statistically processed using nonparametric analysis of dispersions (ANOVA) by the Kruskal—Wallis method and the significant differences between the groups were evaluated using Mann—Whitney method.

RESULTS

The four groups differed significantly by LP_1: H (3, N=37)=17.75858, p=0.0005, LP_2: H (3, N=37)= 10.42004, p=0.0153 and ΔLP values: H (3, N=37)= 15.30825, p=0.0016.

Analysis of LP_1 values showed a significant increase of this parameters in the active control group *vs.* passive control (Z=3.39, p=0.0014; Table 1), which was in line with previous data on reduction of orientation and exploratory activity of animals after injection of the $A\beta_{(25-35)}$ fragment into Meynert basal nucleus [10]. The results in groups 3 and 4 did not differ from those in group 1 and between each other (p>0.1), but differed significantly from group 2 (for group 4 (preventive treatment): Z=3.41, p=0.0006; for group 3: Z=2.78, p= 0.0054). These data indicate that noopept abolished reduction of motor activity, induced by $A\beta_{(25-35)}$ injection into Meynert basal nuclei, by both modes of treatment (Table 1).

A significant reduction of LP_2 was detected in group 2 (active control) in comparison with group 1 (Z=2.64, p=0.008), indicating an amnestic effect of Aβ. In group 4, LP_2 value was significantly higher than in group 2 (Z=2.29, p=0.02) and virtually did not differ from the parameter in group 1. In

TABLE 1. Efficiency of Noopept under Conditions of Preventive and Therapeutic Treatment in Mnestic Deficiency Induced by Injection of $A\beta_{(25-35)}$ in the Basal Nucleus (*M*±*SEM*)

Group	Period of passage into dark compartment, sec		
	LP_1	LP_2	ΔLP
1 (passive control)	10.9±1.8$^+$	128.6±18.9$^+$	117.7±18.9$^+$
2 (active control)	30.4±3.5*	55.6±15.0*	25.2.±15.3*
3 (therapy)	15.1±2.5$^+$	88.6±16.2$^+$	73.4±16.6$^+$
4 (prevention)	10.7±1.1*	108.6±17.6*	97.9±17.6*

Note. $p<0.01$ compared to: *group 1, $^+$group 2.

group 3 LP_2 was also higher than in group 2 (Z= 2.04, p=0.041) and did not differ from that in group 1 (Z=1.61, p=0.108). No appreciable differences were detected between groups 3 and 4 ($p>0.2$), though judging by the means, preventive treatment (group 4) was more effective than therapeutic (group 3).

As our experiments revealed significant differences in LP_1 in different groups, indicating modification of orientation and exploratory activity, we analyzed for evaluation of long-term memory processes, in addition to LP_2, the ΔLP value, characterizing the prolongation of passage of animals into the dark compartment 24 h after training and estimated by the formula $\Delta LP=LP_2-LP_1$. This estimated parameter is more informative under conditions of changed initial behavioral pattern, than the absolute LP_2 duration [10].

Analysis of ΔLP values showed a significant reduction of this parameters in group 2 in comparison with group 1 (Z=3.19, p=0.0014, Fig. 2). In group 4, ΔLP value was significantly higher than in group 2 (Z=2.86, p=0.0042) and virtually did not differ from the parameter in group 1. In group 3 ΔLP was also higher than in group 2 (Z=2.62, p= 0.0089). No appreciable differences were detected between groups 3 and 4 ($p>0.2$), though judging from the means, preventive treatment was more effective than therapeutic.

Thus, this study confirmed previously described failure of CPAR reproduction after bilateral injection of $A\beta_{(25-35)}$ in Meynert basal nuclei of rats [8,10]. Experiments showed that neurodegenerative changes in Meynert nucleus, frontal cortex, and hippocampus start manifesting 1 week after $A\beta_{(25-35)}$

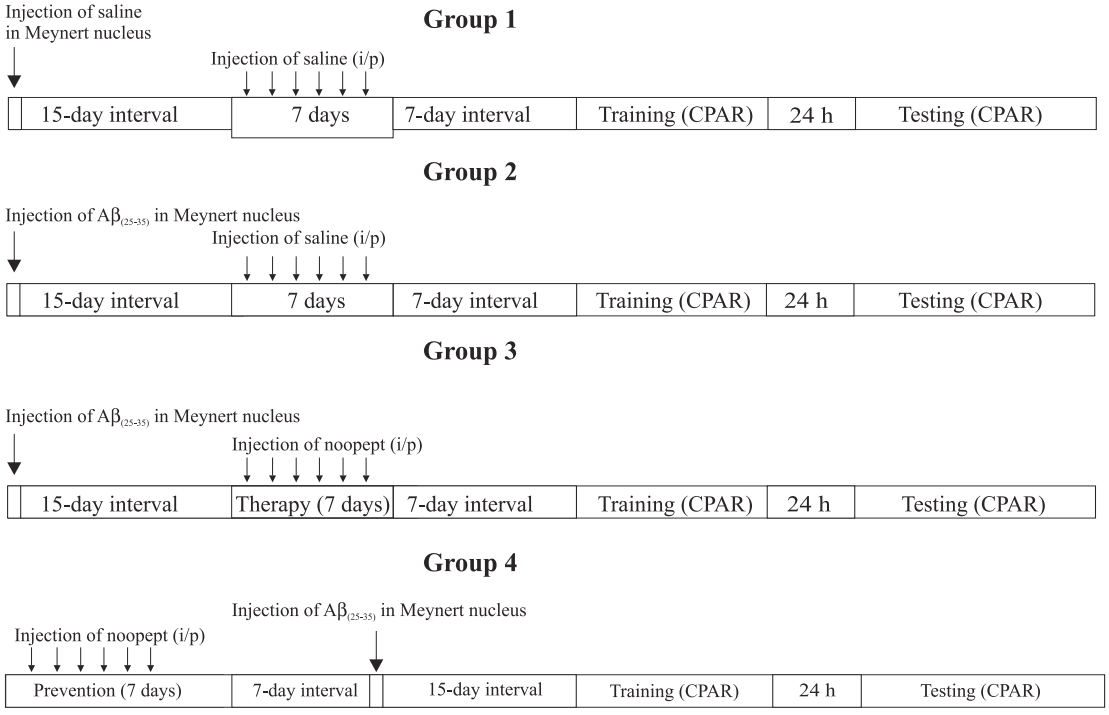

Fig. 1. Scheme of the experiment for each group.

injection and persist for up to 4 weeks. This explains a pronounced memory deficiency during testing 4 weeks after injection in our experiment. It was found that prophylactic injections of noopept prevented the development of amnestic effect of the amyloid. Delayed normalizing aftereffect of noopept is worthy of note: the interval between the last injection of the drug and testing was 24 days. A less pronounced, but clear-cut normalizing effect of noopept on the capacity to extract memory traces was noted after its therapeutic course, started 15 days after damage. The data indicate that noopept in this therapeutic model is characterized by a large "therapeutic window".

Several factors should be taken into consideration in the analysis of the possible mechanisms of noopept effect under these experimental conditions. Injection of $A\beta_{(25-35)}$ into Meynert giant cell basal nuclei leads to reduction of the count of cholinergic neurons and of acetylcholine release by the prefrontal cortical and hippocampal neurons [8,10]. This treatment causes a reduction of cholinacetyltransferase activity [14]. We showed previously that noopept eliminated the amnestic effect of chronic cholinergic deficit. Experiments on isolated neurons of *helix pomatia* showed that this dipeptide, used in a wide range of concentrations (10^{-6}-10^{-11} M) stimulated the reaction to microiontophoretic delivery of acetylcholine to the neuron [3]. As noopept does not modify activity of acetylcholinesterase, presumably its cholinopositive effect is realized at the postsynaptic level. The cholinosensitizing effect of noopept can be a cause of its efficiency towards mnestic deficiency caused by attenuation of cholinergic processes during $A\beta_{(25-35)}$ treatment. However, the mechanism of Aβ neurotoxic effect is not confined to the development of cholinergic deficiency. Amyloid causes a complex of metabolic changes, provoking neurodegenerative processes. This complex includes LPO activation, stimulation of neurotoxic effects of stimulatory amino acids, accumulation of calcium and proinflammatory cytokines [5]. Analysis of the effects of noopept indicates a neuroprotective effect of the drug due to normalization of these metabolic shifts: noopept attenuates LPO [13], blocks potential-dependent K and Ca channels [7], suppresses presynaptic release of glutamate [4]. A pronounced antiinflammatory effect of noopept was detected [1]. Noopept stimulation of production of antibodies to $A\beta_{(25-35)}$ (which can also lead to attenuation of its neurotoxic effect), detected on the model of olfactory bulbectomy [12], is also worthy of note within the framework of these studies.

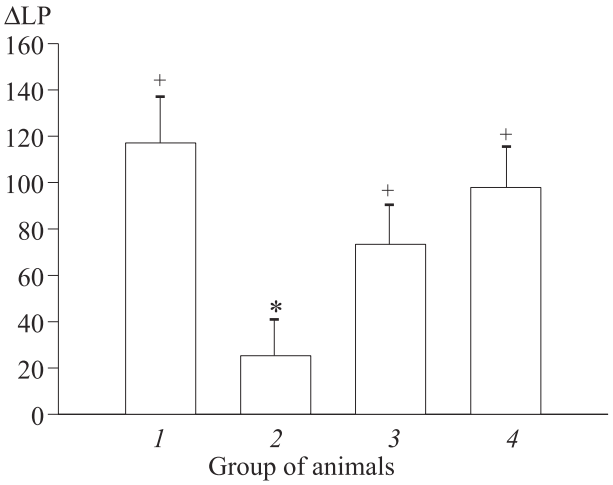

Fig. 2. Efficiency of noopept in experimental AD, induced by injection of $A\beta_{(25-35)}$ into Meynert basal nuclei of rats. $p<0.01$ *vs.* *group 1, +group 2.

The sum of the above-listed nootropic and neuroprotective effects of noopept, its efficiency in some of AD models, its high bioavailability and low toxicity [2] prompt further studies of this systemic active dipeptide as a potential drug for the treatment of AD (initial and moderate stages).

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