# Hexapeptides HLDF-6 and PEDF-6 Restore Memory in Rats after Chronic Intracerebroventricular Treatment with β-Amyloid Peptide Aβ(25-35)

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Effects of homologous peptides HLDF-6 and PEDF-6 on behavior of animals with experimental Alzheimer's disease induced by chronic intracerebroventricular administration of β-amyloid peptide Aβ(25-35) were studied in the zoosocial recognition test and Morris water maze. Peptides HLDF-6 and PEDF-6 possessed neuroprotective activity and counteracted the toxic effect of Aβ(25-35). Peptides HLDF-6 and PEDF-6 mainly improved long-term memory and working memory, respectively.

**Key words:** *peptide Aβ(25-35); peptide HLDF-6; peptide PEDF-6; learning; memory*

Alzheimer's disease is a prevalent neurodegenerative diseases in humans. This disease is characterized by the formation of senile plagues consisting of β-amyloid peptide Aβ(1-40, 1-42) aggregates [12] in the cerebral cortex and hippocampus. The process stimulates several oxidative and toxic reactions damaging brain tissue.

The brain is highly sensitive to oxidative stress. Oxidative stress accompanies inhibition of mitochondrial function, which is observed in Alzheimer's disease [5]. Cytochrome oxidase is a major mitochondrial enzyme involved in aerobic energy metabolism and ATP synthesis. Functional activity of cytochrome oxidase is impaired in patients with Alzheimer's disease [5,13]. Much attention was given to studying the effect of cytochrome oxidase inhibition on memory and learning in animals. Chronic administration of a selective cytochrome oxidase inhibitor sodium azide is an adequate model of Alzheimer's disease [3]. Previous studies showed that inhibition of cytochrome oxidase impairs long-term memory in the Morris water maze test. The impairment of energy metabolism is accompanied by changes in processing of β-amyloid protein precursor, which reduces secretion of non-amyloidogenic peptide fragments and leads to accumulation of β-amyloid peptides [4].

Homologous peptides TGENHR (HLDF-6) and TQVEHR (PEDF-6) are bioactive fragments of differentiation factors HLDF (human leukemia differentiation factor) and PEDF (pigment epithelium-derived factor). Previous studies on rats showed that they protect cerebellar Purkinje cells from degeneration under conditions of sodium azide-induced hypoxia [1].

Here we studied the protective effect of peptides during intracerebroventricular administration of β-amyloid peptide Aβ(25-35) (experimental Alzheimer's disease) [5].

## MATERIALS AND METHODS

Experiments were performed on male Wistar rats aging 120-130 days and weighing 300-400 g. The

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animals were obtained from the Laboratory of Biological Studies (Branch of the M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Pushchino). The rats were kept in cages (4 animals per cage) at 21±1°C and 14:10-h light/dark regimen with free access to food and water. Experimental procedures were conducted according to the requirements of the International Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

The animals were divided into 4 groups (1 control group and 3 experimental groups, 8 rats per group). Rats of experimental groups received peptide Aβ(25-35) alone (group 1) or in combination with HLDF-6 (group 2) or PEDF-6 (group 3).

The test peptides were synthesized as described elsewhere [1]. The solution of peptide Aβ(25-35) in sterile bidistilled water (2 mg/ml) was incubated at 37°C for 4 days to obtain fibrillar aggregates. Light microscopy revealed the presence of fibrillar structures and globular aggregates.

The suspension of Aβ(25-35) was administered intracerebroventricularly for 3 days using an Alzet 1003D osmotic minipump (Alza). Minipumps were unilaterally introduced into the left (50% rats) or right lateral cerebral ventricle (50% rats) of chloral hydrate-anesthetized animals (400 mg/kg) according to stereotaxic coordinates (AP=-1.2, L=±1.5, H=-4.0). The total volume of the Aβ(25-35) suspension was 20 μl. Control rats received an equivalent volume of physiological saline.

HLDF-6 and PEDF-6 were dissolved in physiological saline (50 μg/ml) and injected intramuscularly to rats of experimental groups 2 and 3, respectively. The animals received 8 injections (24 h before pump insertion; 30 min, 24 h, and 3, 5, 9, 14, and 18 days after the end of Aβ(25-35) treatment). The solution was injected in a dose of 1 ml/kg. The rats of experimental group 1 received physiological saline.

The Morris water maze was a round pool (diameter 132 cm, height 60 cm) [15] with white bottom and walls filled to a height of 40 cm with water containing 3 g/liter dry milk (23±2°C). A transparent Plexiglas platform (9×9 cm) was put in the center one quadrants. The platform was placed at a distance of 2 cm from the water surface. The maze was situated in a room with numerous environmental stimuli (cases, calendar, bookcases, *etc.*).

The rats were tested in the water maze on days 25-26 and 85-86 after the end of intracerebroventricular treatment with Aβ(25-35). Water maze testing was conducted for 2 days at a 24-h interval. Each test consisted of 6 trials. The rat was placed in 6 randomly selected points of the pool. The rat snout was directed to the wall of the maze. After reaching the platform the animal was left on it for 30 sec and returned to home cage for 30 sec until the next trial. We recorded the time over which the rat reached the platform (accuracy 0.5 sec).

The results are expressed as *M*±*SEM*. Intergroup differences were evaluated by analysis of variance followed by Newman—Keuls test.

Zoosocial memory was tested on days 20-21 and 80-81 after the end of treatment with peptide Aβ(25-35). Testing was performed by the original method. At stage I we determined the ability of rats to distinguish cagemates from strangers. The rats of the same experimental group were maintained in 2 cages (4 specimens per cage, 8 animals). They were placed in the open filed (diameter 120 cm) for 20 min. The animal's back was labeled with picric acid. We recorded the number and duration of episodes, when the rat was in contact with rats from the same or another cage (sniffing, reciprocal grooming, and aggressiveness). The general ratio between the number of contacts between the rats from the same cage (S) and different cages (D) is the recognition index (S/D=3/4=0.75). The estimated values of S/D were compared with the general ratio of 0.75 (Statistica software). Significant differences between the estimated value and 0.75 reflected the nonrandom nature of contacts (*i.e.*, recognition of neighbors and strangers).

Home cages were interchanged 1 day after the end of test I. The animals from the same cage were placed in different cages with other cagemates. The animals of the same group were tested in the open field 24 h after rearrangement (8 specimens). We estimated the number and duration of various contacts. The S/D ratio was calculated to determine whether the rats could remember their cagemates after isolation.

## RESULTS

On days 25 and 26 after treatment with peptide Aβ(25-35) the rats need more time to reach the platform in the Morris water maze (compared to control animals on days 1 [$F(14, 1)=5.13$, $p<0.05$] and 2 of learning [$F(14, 1)=4.3$, $p<0.05$]). In rats receiving peptide Aβ(25-35) in combination with HLDF-6 or PEDF-6 the mean time of attaining the platform did not differ from the control, but was much shorter compared to Aβ(25-35)-treated animals ($F(14, 1)=3.9$, $p<0.05$; and $F(14, 1)=4.1$, $p<0.05$, respectively). On day 2 of learning the rats injected with peptide Aβ(25-35) and HLDF-6 demonstrated better maze performance compared to Aβ(25-35)-treated animals ($F(14, 1)=6.3$, $p<0.05$). The ani-

mals injected with peptide Aβ(25-35) lost the skill on day 2 of learning (Fig. 1, *a*).

Memory disorders in rats of the Aβ(25-35) group were revealed on day 86, but not on day 85 of study ($F(14, 1)=5.4$, $p<0.05$). Administration of peptides HLDF-6 and PEDF-6 improved maze performance (Fig. 1, *b*).

These data suggest that treatment with peptide HLDF-6 partially restored working memory in animals on day 25 after administration of Aβ(25-35). Moreover, this peptide improved long-term memory on days 26 and 86. Peptide PEDF-6 improved spatial working memory and long-term memory on days 25 and 86 after administration of Aβ(25-35), respectively.

Test I for zoosocial recognition revealed no intergroup differences in the S/D ratio on days 20 and 80 after administration of Aβ(25-35). This ratio was much lower than 0.75. Therefore, the animals more actively contacted with strangers than with cagemates (Tables 1 and 2). Test II for zoosocial recognition was performed 24 h after rearrangement. On days 80 and 81 after administration of peptide Aβ(25-35), the S/D ratio in rats injected with Aβ(25-35) alone or in combination with PEDF-6 significantly exceeded the control. The S/D ratio in these animals approached 0.75. The mean index of zoosocial recognition in rats receiving Aβ(25-35) and HLDF-6 did not differ from the control and was below 0.75 (Table 2). Hence, chronic administration of Aβ(25-35) impaired zoosocial memory 24 h after isolation. These changes were progressive and developed on day 80, but not on day 20 after the start of treatment. Thus, administration of peptide HLDF-6, but not PEDF-6 restored zoosocial memory impaired by Aβ(25-35).

Our results show that peptides HLDF-6 and PEDF-6 possess neuroprotective activity and counteract the toxic effect of β-amyloid fragment Aβ(25-35) on behavioral characteristics of animals. Peptide HLDF-6 mainly protects long-term memory, while peptide PEDF-6 improves working memory. Therefore, the protective effects of these peptides are mediated by various mechanisms.

Neuroprotective activity of peptide HLDF-6 is probably associated with its influence on the liquid crystal structure of lipid membranes and modulation of intracellular cAMP concentration [1]. Membranes are the target of oxidative stress. Lipid composition of membranes is characterized by age-related changes, which underlies the pathogenesis of sporadic Alzheimer's disease. This disorder is manifested in a decrease in the concentrations of phosphatidylethanolamine and phosphatidylinositol in brain membranes [14]. The selective loss of these lipids results from oxidative stress. Aβ(25-35) dose-dependently increases membrane viscosity in brain tissue. Changes in the lipid composition of cell membranes impair intracellular signal transduction and determine progression of neurodegeneration [10]. Aβ(25-35) increases intracellular cAMP concentration in cultured hippocampal neurons [11]. The increase in intracellular cAMP concentration under the influence of forskolin decreases glucose supply to neurons. Glucose deficiency leads to a decrease in ATP synthesis, depolarization of the cell membrane, accumulation of glutamate in the extracellular space, homeostatic dysfunction in neurons, and neuronal death [9]. Impaired glucose transport and exposure to amyloidogenic peptide fragments of Aβ decrease functional activity of mitochondria and inhibit ATP synthesis [8]. In the

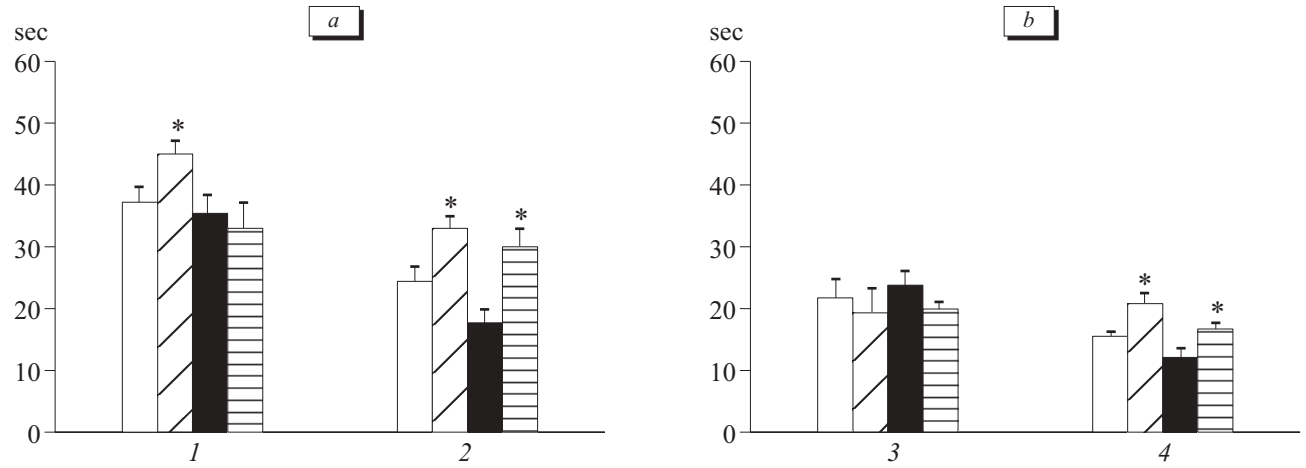


**Fig. 1.** Time of attaining the platform in Morris water maze on days 25-26 (*a*) and 85-86 after administration of Aβ(25-35) (*b*). Days 25 (*1*), 26 (*2*), 85 (*3*), and 86 after administration of Aβ(25-35) (*4*). Light bars, control; slant shading, Aβ(25-35); dark bars, Aβ(25-35) and HLDF-6; horizontal shading, Aβ(25-35) and PEDF-6. *$p<0.05$ compared to the control.

**TABLE 1.** Zoosocial Recognition Indexes in Rats on Days 20 and 21 after Chronic Intracerebroventricular Administration of β-Amyloid Peptide Aβ(25-35)

| Group | Recognition index | | Total number of contacts | |
|---|---|---|---|---|
| | before rearrangement | after rearrangement | before rearrangement | after rearrangement |
| Control | 0.432* | 0.438* | 53 | 58 |
| Experimental group 1 | 0.379* | 0.394* | 92 | 101 |
| Experimental group 2 | 0.418* | 0.437* | 56 | 67 |
| Experimental group 3 | 0.484* | 0.401* | 61 | 117 |

**Note.** *$p<0.05$ compared to the general ratio of 0.75.

**TABLE 2.** Zoosocial Recognition Indexes in Rats on Days 80 and 81 after Chronic Intracerebroventricular Administration of β-Amyloid Peptide Aβ(25-35)

| Group | Recognition index | | Total number of contacts | |
|---|---|---|---|---|
| | before rearrangement | after rearrangement | before rearrangement | after rearrangement |
| Control | 0.406* | 0.431* | 65 | 71 |
| Experimental group 1 | 0.389* | 0.615+ | 48 | 39 |
| Experimental group 2 | 0.434* | 0.457* | 39 | 47 |
| Experimental group 3 | 0.384* | 0.705+ | 51 | 41 |

**Note.** $p<0.05$: *compared to the general ratio of 0.75; +compared to the control.

early and late stage of Alzheimer's disease the rate of ATP synthesis decreases by 7 and 20%, respectively [6]. Neuronal sensitivity to glutamate toxicity and oxidative damage increases during these metabolic disturbances.

Peptide PEDF-6 has no effect on viscosity of lipid membranes. However, this peptide inhibits phosphatidylinositol-specific phospholipase C (PI-PLC) upon its stimulation with aluminum tetrafluoride [1]. The substances activating hydrolysis of phosphatidylinositol phosphates (*e.g.*, amphetamine) impair working memory. Inhibitors of the phosphoinositol cycle (*e.g.*, lithium and sodium valproate) improve working memory after treatment with amphetamine [2]. Peptide Aβ(25-35) 3-fold increases PI-PLC activity in membranes isolated from nerve tissue of human frontal cortex. Moreover, Aβ(25-35) significantly increases intracellular $Ca^{2+}$ concentration [7].

Our findings suggest that homologous peptides HLDF-6 and PEDF-6 produce a neuroprotective effect on brain tissue and counteract the toxic influence of β-amyloid fragment Aβ(25-35).

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