Neuroprotective Effect of Dipeptide AVP(4-5)-NH_2 is Associated with Nerve Growth Factor and Heat Shock Protein HSP70

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In vitro experiments demonstrated the neuroprotective effect of dipeptide pGlu-Asn-NH_2, which corresponded to the N-terminal fragment of the major vasopressin metabolite AVP(4-9). The dipeptide in concentrations of 10^{-5}-10^{-7} M prevented death of HT-22 immortalized hippocampal neurons under conditions of oxidative stress and protected PC-12 rat pheochromocytoma cells from neurotoxic compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. pGlu-Asn-NH_2 in a concentration of 10^{-6} M increased the content of endogenous neuroprotective substances, neurotrophin NGF and heat shock protein HSP70 in HT-22 cells. Our results indicate that this dipeptide can be used for the therapy of Parkinson's disease.

Key Words: *AVP(4-5); dipeptide; neuroprotective activity; NGF; HSP70*

Pituitary hormone arginine-vasopressin (AVP) is a neuropeptide modulating learning and memory. Products of partial AVP proteolysis lose hormonal activity, but retain mnemotropic properties [1,2]. [pGlu^4,Cyt^6]Arginine-vasopressin(4-9) (AVP(4-9) and [pGlu^4,Cyt^6]arginine-vasopressin(4-8) (AVP(4-8) exhibit the highest nootropic activity. In view of low bioavailability of oligopeptides, the search for short dipeptide and tripeptide fragments of AVP crossing the blood-brain barrier and gastrointestinal barrier is an urgent problem.

N-terminal dipeptide fragment of AVP(4-9) (amide of L-pyroglutamyl-L-asparagine, AVP(4-5)-NH_2) was synthesized at the V. V. Zakusov Institute of Pharmacology. Intraperitoneal injection of this dipeptide in doses of 0.01-1.00 mg/kg had a positive mnemotropic effect on rats during passive avoidance conditioning [4].

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Published data show that the content of mRNA for neurotrophins NGF (nerve growth factor) and BDNF (brain-derived neurotrophic factor) in rat cortex and hippocampus increases 12 h after administration of AVP(4-8) [10]. These compounds are endogenous neuroprotective substances. We hypothesized that the dipeptide fragment of AVP(4-5)-NH_2 has neuroprotective activity.

Here we studied the effect of AVP(4-5)-NH_2 on death of HT-22 immortalized hippocampal neurons under conditions of oxidative stress, death of PC-12 rat pheochromocytoma cells after treatment with neurotoxic compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and content of neurotrophin NGF and heat shock protein HSP70 in HT-22 neurons.

MATERIALS AND METHODS

Experiments were performed on cultured HT-22 neurons (immortalized mouse hippocampal cells) and PC12 cells (pheochromocytoma of rat adrenal cortex).

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AVP(4-5)-NH_2 was synthesized at the V. V. Zakusov Institute of Pharmacology (229°C melting point and 14° specific optical rotation angle for 1% aqueous solution).

AVP(4-5)-NH_2 in final concentrations of 10^{-5}-10^{-8} M was added 24 h before and immediately after exposure to destructive factors to evaluate its neuroprotective effect.

The neuroprotective effect of AVP(4-5)-NH_2 against oxidative stress was studied on cultured HT-22 cells. Oxidative stress was induced by H_2O_2 in a final concentration of 1.5 mM [6]. The cells were incubated with H_2O_2 at 5% CO_2 and 37°C for 30 min. The culture medium containing H_2O_2 was replaced with normal medium. Cell viability was evaluated after 4 h.

For modeling of Parkinson's disease, PC12 cells were treated with MPTP in a final concentration of 1 mM [9]. MPTP was added to the cell medium 24 h after cell passage. Cell viability was evaluated 24 h after addition of the toxin.

Cell viability was estimated in the MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) [7]. Optical density was measured on a Multiscan EX spectrophotometer (Thermo) at 600 nm.

The effect of AVP(4-5)-NH_2 (10^{-6} M) on the content of NGF and HSP70 in HT-22 cells was studied by the method of Western blotting [8] with monoclonal antibodies (Santa Cruz Biotechnology) 15 or 24 h after addition of the dipeptide (Fig. 1).

The results were analyzed by Student's *t* test.

RESULTS

Cell viability significantly decreased after addition of H_2O_2 to the cell culture. Addition of AVP(4-5)-NH_2 (10^{-5}-10^{-7} M) 24 h before stress was followed by partial recovery of neuronal viability. Addition of AVP(4-5)-NH_2 in the same concentrations immediately after removal of H_2O_2 produced a more potent protective effect (Table 1). The dipeptide in a concentration of 10^{-8} M was ineffective in both cases. Our results indicate that AVP(4-5)-NH_2 produced a delayed direct neuroprotective effect during oxidative stress.

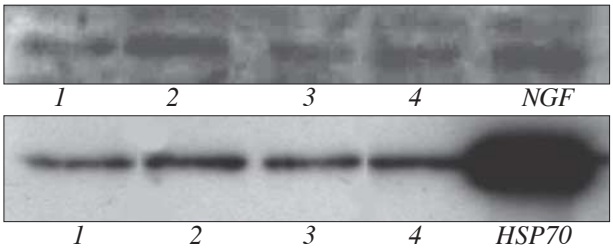

Fig. 1. Content of NGF (*a*) and HSP70 (*b*) in cultured HT-22 hippocampal neurons 24 (*2*) and 15 h (*4*) after addition of AVP(4-5)-NH_2 (10^{-6} M) to the culture medium (original Western blotting). Culturing of cells for the same period of time, control for *2* (*1*) and *4* (*3*). Here and in Fig. 2: results of 3 independent experiments.

MPTP decreased neuronal viability by 20-40%. Addition of AVP(4-5)-NH_2 in concentrations of 10^{-5}-10^{-8} M to the culture of PC12 cells 24 h before MPTP treatment produced a potent protective effect. However, addition of the dipeptide in various final concentrations immediately after MPTP treatment was ineffective (Table 1).

NGF is an endogenous neuroprotective compound. AVP metabolite AVP(4-8) increases the synthesis of mRNA for NGF in the hippocampus. Since AVP(4-5)-NH_2 is a N-terminal dipeptide fragment of nootropic AVP metabolites, its protective effect can be related to activation of NGF synthesis in hippocampal neurons. To evaluate the molecular mechanism of neuroprotective activity of AVP(4-5)-NH_2, we studied the effect of this compound on the content of NGF and HSP70 in the culture of HT-22 immortalized hippocampal neurons. The dipeptide was added in a final concentration of 10^{-6} M. AVP(4-5)-NH_2 in this concentration was most

TABLE 1. *In Vitro* Neuroprotective Effect of Dipeptide AVP(4-5)-NH_2 (Survived Neurons, % of the Control, *M*±*m*)

Model	Time of AVP(4-5)-NH_2 addition				
	without AVP(4-5)-NH_2	AVP(4-5)-NH_2, M			
		10^{-5}	10^{-6}	10^{-7}	10^{-8}
Oxidative stress (HT-22 cells)					
24 h before H_2O_2	66±5*	83±19+	75±10+	75±8+	72±11
simultaneously with H_2O_2	82±10*	112±12+	119±6+	124±12+	102±36
Parkinson's disease (PC12 cells)					
24 h before MPTP	80±10*	100±7+	95±6+	96±7+	97±9+
simultaneously with MPTP	64±11*	72±10	70±9	65±2	65±9

Note. $p<0.05$: compared to the control *without damaging agent; +with damaging agent.

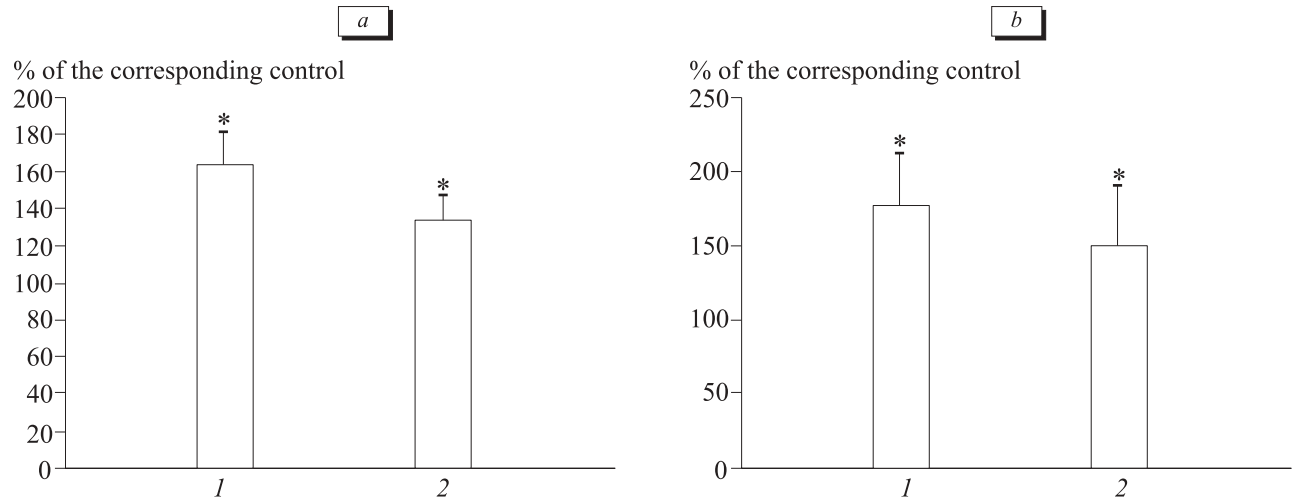

Fig. 2. Content of NGF (*a*) and HSP70 (*b*) in the culture of HT-22 hippocampal neurons 24 (*1*) and 15 h (*2*) after addition of AVP(4-5)-NH_2 (10^{-6} M) to the culture medium (densitometry). Parameters of the corresponding control (cell culturing for the same period of time) are taken as 100%. *$p<0.05$ compared to the corresponding control.

effective on the model of cell damage with H_2O_2 and MPTP. NGF content increased by 33 and 64% after addition of AVP(4-5)-NH_2 15 and 24 h before analysis, respectively (Fig. 2). HSP70 content significantly increased 15 and 24 h after addition of the dipeptide (by 50 and 44%, respectively; Fig. 2).

The delayed effect of this dipeptide is probably associated with increased expression of endogenous neuroprotective compound NGF and protective heat shock protein HSP70. HSP70 is a molecular chaperone involved in renaturation of damaged proteins. Due to chaperone activity, HSP70 can inhibit the formation of α-synuclein fibrils during Parkinson's disease and β-amyloid during Alzheimer's disease [3,5]. Apart from the decrease in the content of neurotrophins, accumulation of these substances contributes to the pathogenesis of neurodegenerative diseases. Further *in vivo* studies are required for evaluation of the neuroprotective effect of AVP(4-5)-NH_2 as a compound stimulating endogenous defense systems in neurons.

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