Morphochemical Changes in Brain Structures and Their Correction by Tuftsin in Rats with Hypofunction of the Dopamine System

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Chronic administration of haloperidol to Wistar rats induces specific changes in protein metabolism at the cortical-subcortical level (sensorimotor cortex—caudate nucleus), in associative and integrative triggering neurons, and in the cytoplasm and nucleus of the same neuron, judging from the state of structural proteins and aminopeptidase activity. Tuftsin reduces these changes only in the sensorimotor cortex.

Key Words: *sensorimotor cortex; caudate nucleus; protein metabolism; haloperidol; tuftsin*

Numerous pathologies of the central nervous system (CNS) are associated with changes in the state of the catecholaminergic system, specifically, in the brain content of dopamine [8,9]. The haloperidol model makes it possible to study the CNS function when the dopamine system is hypoactive. Physiological studies showed that chronic administration of haloperidol results in behavioral changes typical of bradykinesia attended by impaired motor integration [2,6].

The present study was designed to examine changes in responses of some brain structures under conditions of so-called "dopamine pathology" and explore the possibility of correcting these changes with the neuropeptide tuftsin. Tuftsin is a nonspecific immunostimulating agent which elicits a central effect on the behavior of animals. Intact animals develop general motor excitation within the first 30 min after administration of tuftsin [4].

MATERIALS AND METHODS

The study was carried out on adult male Wistar rats weighing 200-250 g. Control rats received physiological saline throughout the experimental period (group 1). Group 2 consisted of rats injected with haloperidol (intraperitoneally, daily dose 0.5 mg/kg body weight) every day for 4 weeks, and group 3 included haloperidol-treated rats given a single injection of tuftsin (300 mg/kg body weight intraperitoneally) 60 min after the last haloperidol injection.

All rats were decapitated under light ether anesthesia 40-60 min after the last injection. The sensorimotor cortex (layers III and V) and caudate nucleus were removed for study. One cerebral hemisphere was fixed in Carnoy's fluid for subsequent embedding in paraffin and interferometry, while the other was frozen, after which 20-μ sections were cut in a cryostat for the aminopeptidase (AMP) activity assay. The protein content in neuronal cytoplasm and nuclei of the studied structures was measured by interferometry in monochromatic light at 535 nm using an Interfaco microscope. The major and minor axes of the ellipse outlining the cells and nuclei were measured with the ocular micrometer, and the areas of their profile fields were calculated. The data were processed using a *Protein* software developed at the Laboratory of Cytochemistry (Institute for Brain Research). The AMP activity was measured in cryostat sections using leucyl-2-naphthylamide as a substrate [1] and expressed in arbitrary optical density units

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as estimated under a LYuMAM-I3 microscope at λ=550 nm.

RESULTS

Chronic administration of haloperidol did not alter the size of the layer III neurons (the major cell type responsible for intracortical connections), but increased the protein content and concentration in their cytoplasm compared with control levels. Neuronal cytoplasm of haloperidol-treated rats given a single injection of tuftsin shrunk, while their protein concentration increased. The cytoplasmic protein content did not differ considerably from that in haloperidol-treated rats.

The layer III neurons from haloperidol-treated rats had smaller nuclei and contained lesser amounts but higher concentrations of proteins. Tuftsin normalized the size of neuronal nuclei and increased the protein content to levels considerably higher than in haloperidol-treated and control rats. These morphochemic changes indicate that protein synthesis predominates over protein degradation [3,5].

In haloperidol-treated rats, all parameters of the cytoplasm and nuclei of the layer V neurons, which perform the efferent-projection function and form an extensive network of axodendritic connections, were lower than in the control. This probably indicates that protein degradation predominates over protein synthesis. Tuftsin normalized the size of the nucleus and cytoplasm and elevated both the protein content and concentration higher than in control rats (Table 1). Therefore, it can be concluded that the haloperidol-induced inhibition of protein synthesis in the layer V neurons was abolished by tuftsin.

TABLE 1. Aminopeptidase (AMP) Activity and Protein Content and Concentration (Dry Matter) in Neurons of the Sensorimotor Cortex and Caudate Nucleus After a Single Administration of Tuftsin to Haloperidol-Treated Rats

Parameter	Sensorimotor cortex						Caudate nucleus		
	layer III			layer V					
	control	haloperidol	haloperidol+ tuftsin	control	haloperidol	haloperidol+ tuftsin	control	haloperidol	haloperidol+ tuftsin
Cytoplasm									
AMP activity×10^{-2}	29.30±0.20	38.3±0.20	32.4±0.30	42.6±0.20	43.9±0.20	42.8±0.20	36.9±0.20	38.3±0.20	39.5±0.20
	(100)	(130.7)	(110.6)	(100)	(103.1)	(100.5)	(100)	(103.8)	(107)
Area, μ^2	60.65±0.78	60.40±0.98	57.69±0.63	196.38±3.39	157.90±2.85	197.40±3.51	37.54±0.26	36.16±0.50	34.21±0.32
	(100)	(99.6)	(95.1)	(100)	(80.4)	(100.5)	(100)	(96.3)	(91.1)
Protein content, pg	97.04±1.54	101.80±2.65	102.29±1.65	362.5±8.78	270.51±6.52	428.94±11.21	56.13±0.65	57.72±1.12	51.87±0.55
	(100)	(104.1)	(105.4)	(100)	(74.6)	(118.3)	(100)	(104.6)	(92.4)
Protein concentration, pg/μ^3	1.60±0.01	1.67±0.03	1.77±0.02	1.83±0.02	1.7±0,02	2.15±0.03	1.49±0.01	1.62±0.02	1.52±0.01
	(100)	(104.4)	(110.6)	(100)	(92.9)	(117.5)	(100)	(108.7)	(102)
Nucleus									
Area, μ^2	94.7±1.09	81.36±0.80	94.75±1.00	165.32±1.98	126.88±1.77	161.87±1.58	67.93±0.68	59.16±0.68	68.17±0.76
	(100)	(85.9)	(100.1)	(100)	(76.7)	(97.9)	(100)	(87.1)	(100.4)
Protein content, pg	70.71±1.31	66.56±1.71	84.78±2.60	156.35±3.61	98.74±2.12	170.36±4.03	58.00±0.85	45.77±0.86	50.49±0.70
	(100)	(94.1)	(119.9)	(100)	(63.2)	(109)	(100)	(78.9)	(87.1)
Protein concentration, pg/μ^3	0.75±0.01	0.82±0.02	0.89±0.02	0.94±0.01	0.78±0.01	1.05±0.02	0.75±0.01	0.77±0.01	0.75±0.01
	(100)	(109.3)	(118.7)	(100)	(83)	(111.7)	(100)	(102.7)	(100)

Note. The percent is given in parentheses.

Neurons forming the caudate nucleus, which is known to inhibit motor functions and the activity of other extrapyramidal structures, responded to haloperidol by a decrease in the size of their cytoplasm and nuclei and an increase in the protein content and concentration. After administration of tuftsin, the cytoplasm shrunk more. The protein concentration became almost normal, while the protein content dropped in comparison with both haloperidol-treated and control rats. The protein content in the nuclei of the caudate nucleus neurons markedly decreased in haloperidol-treated rats, being partially restored by tuftsin, which normalized the size of the nuclei. The protein concentration in the nuclei of the caudate nucleus neurons was virtually the same in the three groups (Table 1).

Histochemically determined activity of AMP (a protein-degrading enzyme) was considerably higher in layer III of the sensorimotor cortex of haloperidol-treated rats and much lower in the cortex of rats injected with tuftsin after haloperidol treatment. There were no differences in AMP activity in other brain structures compared with the control (Table 1).

Our results show that differences in the response to chronic haloperidol treatment are manifested at three levels: 1) neurons of cortical and subcortical structures, 2) neurons of different morphofunctional types within the cortex, and 3) cytoplasm and nucleus of the same neuron. Tuftsin induces different reactions mainly at the level of cortical and caudate nucleus neurons. Thus, it corrects changes caused by chronic haloperidol treatment in cortical neurons but has little effect on changes occurring in caudate nucleus, particularly those in the neuronal cytoplasm. Tuftsin eliminates bradykinesia and induces specific bioelectrical activity in the motor cortex and caudate nucleus [7].

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