MORPHOLOGY AND PATHOMORPHOLOGY

Cytochemical and Morphological Changes in Cerebral Neurons Induced by δ-Sleep Peptide

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Small neurons prone to chromophilia, with protein production decreased to 40%, were found in layer V of the sensorimotor cortex of rats with emotional fear induced by antistress δ-sleep peptide. These changes point to sedative effect of δ-sleep peptide in a stimulated focus.

Key Words: *brain; autoradiographic protein production; dopamine system hyperfunction; δ-sleep peptide*

Stress-protective and regulating effects of δ-sleep peptide (DSP) have been extensively studied in various diseases [3-6,10,11] during the last two decades [13]; however, metabolism modifications and restructuring in the brain caused by this peptide are still little known.

We studied cytochemical and morphological changes in cerebral neurons induced by the antistress agent DSP in animals exposed to stress or extreme factors.

MATERIALS AND METHODS

Hyperfunction of the dopamine system causing a complex of behavioral reactions in animals: decreased motor activity, impaired conditioned reflex defense reactions, and emotional fear [1,2,12], was induced in male Wistar rats weighing 180±10 g by daily intraperitoneal injections (for 21 days) of Madopar-125 (L-DOPA) in a dose of 100 μg/kg. Control rats were injected with normal saline. On day 21 the rats were injected with DSP (M. M. Shemyakin Institute of Bioorganic Chemistry) in a dose of 100 mg/kg, and the peptide effect was monitored for 1 h. Protein production in the brain was studied by autoradiography. To this end, L-leucin-[2,3-^{3}H] (specific activity 160 GBq/liter, Izotop) was injected intraperitoneally (44.4 kBq/g) to three groups of rats: intact controls, animals injected with L-DOPA, and with L-DOPA+DSP. After 2.5 h protein production was assessed. A total of 100 cells from 3 rats in each group were examined. Microradioautographs were prepared as described previously [8,9]. Silver grains in neuron radioautographs were counted under a light microscope; the intensity of labeled compound incorporation in neuronal proteins and, hence, the production of protein in a neuron was assessed by the number of silver grains per unit of cell structure area. The morphology of neurons was studied on preparations stained with cresyl violet and hematoxylin and eosin. The size of neuronal corpuscles was measured with an AM9-2 ocular micrometer. The data were statistically processed using Fisher-Student's test.

RESULTS

Administration of L-DOPA in a dose of 100 mg/kg for 21 days stimulated protein production in the rat brain (Table 1). Incorporation of ^{3}H-leucin in layer V of neurons of the sensorimotor cortex and of the

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TABLE 1. Incorporation of ^{3}H-Leucin in Rat Neuronal Proteins after Prolonged Administration of L-DOPA Followed by an Injection of DSP (*M±m*)

Experiments	Intensity of ^{3}H-leucin incorporation, arb. units	Difference from control, %	Difference from experiments with L-DOPA, %	Neuron area, μm² (at ×1500)	Difference from control, %	Difference from experiments with L-DOPA, %
Injection of L-DOPA						
Sensorimotor cortex, V layer						
control	0.146±0.01			436.44±10.31		
L-DOPA	0.189±0.01	+29**		368.39±9.89	-16*	
Caudate nucleus						
control	0.107±0.01			122.24±1.88		
L-DOPA	0.116±0.01	+8*		124.15±1.98	+2	
Injection of L-DOPA+DSP						
Sensorimotor cortex, V layer						
(1)	0.118±0.01		-38**	291.81±3.62		-21**
(2)	0.124±0.01		-34**	225.84±2.66		-39**
Caudate nucleus						
(1)	0.085±0.01		-26**	111.80±1.85		-10*

Note. Slight (1) and moderate (2) contraction of a cell. *p<0.05, **p<0.01 *vs.* control, *p<0.05, **p<0.01 *vs.* L-DOPA.

caudate nucleus was increased. Linear size of neurons did not change in the caudate nucleus and decreased in layer V. No other morphological changes were observed. This agrees with our previous data [8,9]. One hour after injection of DSP, small neurons prone to chromophilia, which we denoted as contracted, appeared in the brain. They were 21-71% smaller than those in animals treated with L-DOPA alone (Tables 1 and 2). By this parameter the neurons were divided into 3 groups: slightly, moderately, and strongly contracted. There were no other abnormalities in these cells; they were located mainly in the sensorimotor cortex and partially in the visual areas, predominantly in layer V with the projection-efferent function and in lesser amounts in layer VI. They were not found in layers II-IV (upper layers with associative and efferent functions). In layer V at an area of 40,000 μm² there were 11 moderately and strongly contracted neurons (Table 3) as compared with one chromophilic neuron per 10-11 areas of the same size in intact controls and rats not injected with DSP. Incorporation of ^{3}H-leucin was similarly decreased (by 34-38%) in slightly and moderately contracted neurons (Table 1). The label incorporation in strongly contracted neurons was almost the same; it was difficult to count silver grains in these cells because of their strong chromophilia.

Thus, decreased area and protein production in these neurons, which are most pronounced in large-cell projection-efferent elements of layer V, can be regarded as characteristic signs of antistress effect of DSP in animals in a state of emotional fear. On the other hand, these morphological and cytochemical signs are well-known indicators of decreased functional activity of neurons. Based on A. A. Ukhtomskii's theory on the dominant, according to which cortical zones containing large pyramidal

Table 2. Effect of DSP on the Area of Large Pyramids in layer V of Sensorimotor Cortex of Rats Treated with L-DOPA (*M±m*)

Morphological changes	Neuron area, μm² (at ×600)	Difference from experiments with L-DOPA, %
L-DOPA		
Neurons unchanges	412.22±4.34	
L-DOPA+DSP		
Moderately contracted neurons	233.92±7.00	-43*
Strongly contracted neurons	119.85±4.35	-71*

Note. *p<0.01 *vs.* L-DOPA alone.

Table 3. Number of Different Types of Neurons in Layer V of Sensorimotor Cortex in Rats Treated with L-DOPA and Then Injected with DSP

Neuron type	Number of neurons per 40,000 μm³ (%)
Strongly contracted neurons	
large	2.06 (18)
medium-sized	1.98 (18)
small	1.83 (16)
Moderately contracted neurons	
large	1.26 (11)
medium-sized	2.83 (25)
small	1.14 (10)

neurons and the possibility of formation in these structures of stable stimulation with the dominant properties are significant [7], we believe that a decrease in protein production and cell size induced by DSP are cytochemical and morphological manifestations of inhibition in a focus of stimulation formed under the effect of prolonged administration of L-DOPA.

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