Immunohistochemical and Morphological Changes in Neurons and Neuroglia in the Cerebral Nigrostriatal Structures under Conditions of Experimental Nigral Neurodegeneration

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The count of dopamine-containing neurons decreased by 77%, the area of the remaining cells shrank by 75%, and the neuroglia doubled 4 weeks after injection of toxin (6-hydroxydopamine) into the compact part of the substantia nigra of the right cerebral hemisphere of rats, while no changes in the substantia nigra of the left hemisphere were observed. Neurons of the caudate nucleus were virtually unchanged in comparison with the intact control, while the neuroglia was activated: its total volume in the right hemisphere increased by 33% (50% increase in astrocyte count and a 25% increase of the rest neuroglia), while in the left hemisphere only astrocyte count increased by 20%. Astrocyte nuclei in the caudate nuclei of both hemispheres were enlarged by 22-23%. Hence, unilateral destruction of the nigral dopamine-containing neurons stimulated the neuroglia (particularly astroglia) in the caudate nuclei, especially on the side of damage.

Key Words: *brain; neuron; neuroglia; dopamine; neurodegeneration*

The neuron-neuroglia interactions now attract special attention of scientists, because they seem to determine the course of many pathological processes in the brain, including neurodegenerative diseases [9,10]. Studies of the pathogenetic bases of neurodegenerative process have demonstrated that neurodegeneration developing in the nigrostriatal structures of the brain destroys not only dopamine-producing neurons, but also disorders the interactions between dopaminergic structures of the brain [15]. On the other hand, the aftereffects of morphological and neurochemical changes developing in the cells of the cerebral dopaminergic formations in humans and animals suffering from these disorders remain little studied [4].

We studied the effect of unilateral destruction of the substantia nigra compact part in the brain of Wistar rats on the morphology and immunohistochemistry of the neurons and neuroglia in the striatum and substantia nigra structures of the ipsilateral and contralateral hemisphere.

MATERIALS AND METHODS

Unilateral degeneration of the substantia nigra was reproduced in male Wistar rats (200-250 g, *n*=4) by injection of neurotoxin (6-hydroxydopamine; 6-OHDA) [8] using a stereotaxic manipulator for small laboratory animals (Stolting). Rats under total anesthesia were injected with 6 μg 6-OHDA dissolved in 3 μl 0.05% ascorbic acid into the compact part of the right hemispheric substantia nigra and with 3 μl 0.05% ascorbic acid into the left hemisphere (active control). The coordinates of stereotaxic injection were as follows: AP=4.2, V=1.9, L=7.0 [11]. Male Wistar rats not subjected to experimental manipulations of any kind (200-250 g, n=4) served as intact controls. Ketamine (50 mg/kg) and benzodiazepine (5 mg/kg) intraperitoneally were used for total anesthesia. Horizontal motor

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activity of experimental and intact rats was studied in the open field test. The intensity of stereotypical reactions was evaluated under the effect of apomorphine (dopamine receptor agonist) injected subcutaneously in a dose of 0.8 mg/kg, its effects on animal behavior were studied over 1 h. The animals were kept and handled in accordance with Laboratory Practice Regulations in the Russian Federation, approved by the Order No. 267 of the Ministry of Health of the Russian Federation, June 19, 2003. Four weeks after injection of 6-OHDA all animals were decapitated under ether narcosis, the brain was fixed in Carnoy fluid, embedded in paraffin, and 7-μ sections were sliced at the level of the dorsomedial region of the caudate nucleus and the compact part of the substantia nigra.

Dopamine-containing structures in the brain (neuronal bodies and axons) were detected by immunohistochemical staining for tyrosine hydroxylase [1]. Immunohistochemical staining for glial fibrillary acidic protein (GFAP) detecting astrocytes was combined with cresyl violet staining to detect the glial cells containing no GFAP. Immunohistochemical reactions were carried out by the avidine peroxidase method (according to Sigma protocol) with rabbit antibodies (first antibodies; Sigma), goat biotinylated antibodies (second antibodies; Sigma, EXTRA-3 Kit) to rabbit immunoglobulins, and 3,3-diaminobenzidene (chromogen). For morphological control, the brain sections were stained by Niessle's method. Morphometry of immunohistochemical and morphological preparations was carried out under a Leica DMLB microscope (×40 objective) fitted with Leica Qwin videoanalysis software [2]. A total of 100 visual fields in the caudate nucleus and 80 in the substantia nigra of each experimental animal were examined. The densities of neurons and total neuroglia population were evaluated and the glioneuronal index was calculated (neuroglia/neuron cell counts), and GFAP-containing astrocytes and astrocytes without GFAP were counted. The areas of neuronal bodies and neuroglia nuclei were measured. The data were processed by StatSoft Statistica 6.0 software.

RESULTS

The horizontal motor activity of experimental rats in the open field progressively decreased under the effect of 6-OHDA on the right hemispheric substantia nigra compact part, and by week 4 the animals developed marked hypodynamia (Fig. 1). Apomorphine functional test (characterizing dopamine neuron injury) showed that the animals rotated to the left at a velocity of more than 30 rpm under the effect of apomorphine. This indicated damage to the dopaminergic neurons in the right hemisphere.

Immunohistochemical staining for tyrosine hydroxylase indicating the presence of dopamine-containing structures in the brain was virtually not detected in the compact part of the cerebral right hemisphere substantia nigra (at the site of 6-OHDA injection), and in the right hemispheric caudate nucleus was less intense than in intact animals.

Studies at the cellular level (computer-aided morphometry) showed that 6-OHDA neurotoxin destroyed 77% dopamine-containing neurons in the compact part of the right hemispheric substantia nigra, while the area of the remaining tyrosine hydroxylase-positive neurons decreased by 75% in comparison with the intact control (Tables 1, 2). On the other hand, the counts and sizes of dopamine-containing neurons in the substantia nigra in the left hemisphere of these animals (active control) were virtually unchanged in comparison with intact rats. The total volume of the neuroglia in the substantia nigra in the right (ipsilateral) hemisphere increased 2-fold in comparison with the intact control and virtually did not change in the left hemisphere (active control). Hence, the glioneuronal (neuroglia/neuron) index in the substantia nigra increased 9-fold in the right hemisphere and did not differ from the intact control in the left hemisphere. Neither the neuron counts, nor their sizes changed in the caudate nuclei of experimental in comparison with intact rats. On the other hand, a stable trend to shrinkage of the neuronal area (by 15%) in the caudate nucleus was observed in the right hemisphere (on the side of 6-OHDA injection). The content of total neuroglia in the caudate nucleus increased by 33% in the right hemisphere and remained unchanged in the left hemisphere. The glioneuronal index in the caudate nucleus of the right hemisphere increased by 32% in comparison with intact control.

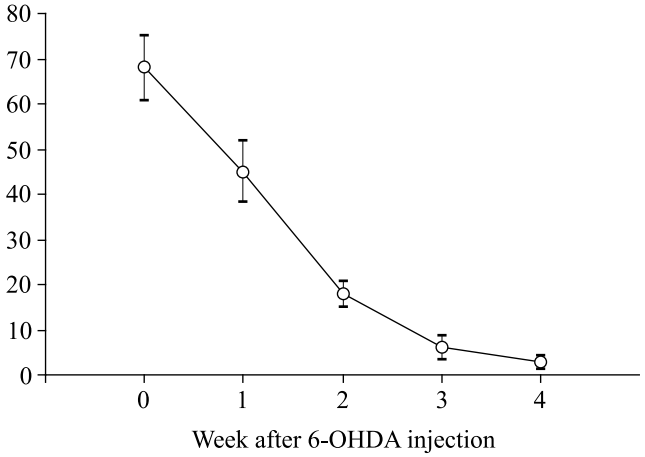

Fig. 1. Changes in the horizontal motor activity of Wistar rats in the open field, evaluated by the number of crossed squares, under the effect of 6-OHDA neurotoxin. Ordinate: number of squares crossed by rats in 3 min.

TABLE 1. Changes in the Density of Neurons and Total Neuroglia in the Compact Part of the Substantia Nigra and Caudate Nuclei of the Right and Left Hemispheres of Wistar Rats in Response to Local 6-OHDA and Its Effects on the Nigral Dopamine-Containing Neurons in the Right Hemisphere

Brain compartment, parameter	Experiment conditions		
	IC	AC	6-OHDA
Substantia nigra			
neurons			
density	6.8±1.1	7.5±1.8	1.6±1.0
difference from IC, %		+10	-77*
total neuroglia			
density	16.2±1.9	18.2±2.6	33.9±5.7
difference from IC, %		+12	+109*
glioneuronal index	2.4	2.4	21
Caudate nucleus			
neurons			
density	22.2±5.6	22.1±3.8	23.0±5.4
difference from IC, %		0	+4
total neuroglia			
density	16.2±4.4	17.2±4.3	21.5±5.5
difference from IC, %		+6	+33*
glioneuronal index	0.73	0.80	0.96

Note. Here and in Table 2: IC: intact control; AC: active control; 6-OHDA: side of 6-OHDA injection. *p<0.05 in comparison with intact control.

Various neuroglia types in the caudate nuclei differently reacted to substantia nigra destruction by 6-OHDA neurotoxin (Fig. 2). Astrocytes detected by the immunohistochemical method (GFAP staining) were most active. Their levels increased by 50% on the side of destruction (in the right caudate nucleus) and by 20% in the left hemisphere in comparison with intact control. Not only the count of astrocytes, but also the size of their nuclei increased: the area of astrocyte nuclei increased by 22-23% in comparison with intact control in both hemispheres. Neuroglial cells ($GFAP^-$) were less active: their counts increased by ¼ in comparison with intact control only in the right hemisphere, while the size of their nuclei did not change.

Immunohistochemical staining for tyrosine hydroxylase used as the marker of dopamine-containing structures of the brain showed that 4 weeks after injection of 6-OHDA neurotoxin into the compact part of the substantia nigra in the right hemisphere 2/3 of neurons of this nucleus died and the remaining cells were partially destroyed, while total neuroglia volume increased 2-fold, which shifted the neuroglia/neuron ratio: the glioneuronal index increased 9-fold. No appreciable changes were detected in neurons or neuroglia of this structure in the left hemisphere (active control), which suggested a sharp stimulation of the neuroglia in the substantia nigra on the side of neurotoxin injection as a reaction of brain structures to neurodegenerative process [3]. Horizontal motor activity of experimental rats tested in the open field decreased 13-fold by week 4 of the experiment in comparison with intact rats; the animals developed severe hypodynamia indicating significant structural and functional changes in the entire system of the cerebral nigrostriatal formations, specifically, in the caudate nucleus, the most important component of this system, suffering from dopaminergic denervation in neurodegenerative processes [5,6,15].

No appreciable changes in the neurons, cell counts, and sizes were detected in the caudate nuclei of both hemispheres of experimental rats. A stable trend to reduction of the caudate nucleus neuron areas (15%) in comparison with intact controls was detected. This could be regarded as reduced functional activity of the caudate nuclei neurons in response to destruction of the dopaminergic terminals, neuronal axons reaching the caudate nucleus from the substantia nigra subjec-

TABLE 2. Changes in the Size of Neurons in the Substantia Nigra Compact Part and Caudate Nuclei of the Right and Left Hemispheres of Wistar Rats 4 Weeks after Local Destruction of the Right Hemispheric Substantia Nigra by 6-OHDA Neurotoxin

Experiment conditions	Substantia nigra		Caudate nucleus	
	neuron size, μ²	difference from IC, %	neuron size, μ²	difference from IC, %
IC	296.0±42.2		117.7±27.3	
AC	322.5±43.0	+9	104.5±30.7	-11
6-OHDA	72.7±3.46	-75*	100.0±28.6	-15

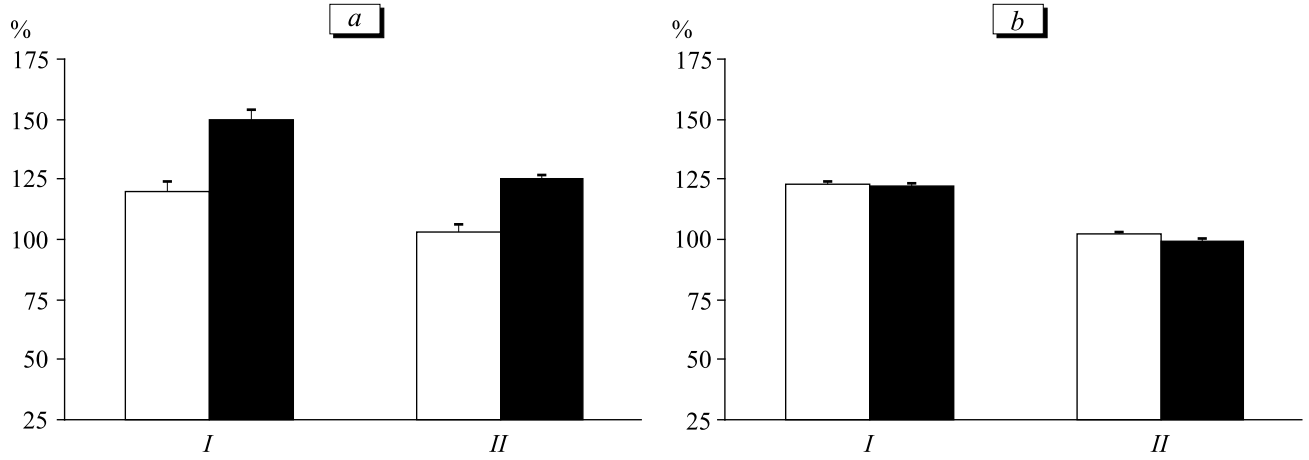

Fig. 2. Morphometric parameters of various neuroglia types in the caudate nuclei of the right and left hemispheres of Wistar rats 4 weeks after local destruction of the substantia nigra compact part by 6-OHDA neurotoxin in the right hemisphere. Ordinate: difference *vs.* intact control (100%). *a*) density of neuroglia in caudate nuclear structures; *b*) neuroglial cell nuclei sizes. *I*) $GFAP^+$ astrocytes; *II*) $GFAP^-$ neuroglial cells. Light bars: active control; dark bars: side of 6-OHDA injection.

ted to destruction [5]. It seemed that denervation processes were also responsible for 1/3 increase of the total neuroglia population in the caudate nucleus on the side of destruction in comparison with intact control.

A differentiated approach to evaluation of various neuroglia types in the caudate nuclei showed the most active response of astrocytes (detected by the immunohistochemical method — GFAP staining) to simulated neurodegeneration of the substantia nigra. The count of these cells increased by 50% in the right and by 20% in the left hemisphere in comparison with intact control. Astrocyte nuclei were similarly enlarged in the right and left hemispheres (by 22-23%), while the $GFAP^-$ neuroglia nuclei did not change, and their count increased by ¼ in comparison with intact control and only on the side of destruction. As the cell nucleus was the central component of cell metabolism, we concluded that the caudate nucleus astrocytes responded most actively to induced neurodegeneration of the substantia nigra: by increase in their count and by more intense reaction of their nuclei. Published data confirmed the important role of astrocytes in the pathophysiology of neurodegenerative processes [7,12,13].

Hence, unilateral destruction of the substantia nigra dopamine-containing neurons stimulated the neuroglia, particularly astroglia, in the caudate nuclei, more so on the side of destruction.

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