BIOPHYSICS AND BIOCHEMISTRY

Structural, Functional, and Biochemical Changes in the Brain during Modeling of Dopamine System Disturbances in Rats

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Dysfunction of the dopamine system was modeled in Wistar rats by injection of 50 mg/kg L-dopa over 4 weeks. Experimental rats demonstrated considerably decreased locomotor activity and increased emotional strain compared to the control group. Structural changes consisted in a significant decrease in the size of neuronal bodies in the sensorimotor cortex (layers III and V) and caudate nucleus together with changed variability of these parameters compared to the corresponding values in the control. The neuroglial index increased by 22% in layer V, tended to decrease in layer III, and remained unchanged in the caudate nucleus. L-Dopa changed specific activity of enzymes: tyrosine hydroxylase activity in the sensorimotor cortex decreased by 25%, while monoamine oxidase B activity in the caudate nucleus increased by 33%. Thus, dysfunction of the dopamine system resulting in changes in dopamine metabolism not only leads to structural and functional rearrangements reducing functional capacities of the cell systems, but is also associated with compensatory and repair reactions in the brain.

Key Words: *brain; neuron; neuroglia; metabolic dysfunction*

Disturbances in neurotransmitter metabolism are an important aspect of the studies of mechanisms underlying the development of nervous and psychic disorders [3,5]. At the same time, structural and functional changes in the brain under these conditions are poorly studied.

Here we studied structural and biochemical changes in various systems of neurons and neuroglial cells of the brain cortex and subcortical formations during modeling of dopamine system hyperfunction.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 180±20 g. The animals of the experimental and control groups (each comprised 11 rats) received daily intraperitoneal injections of Madopar-125 (L-3,4-dihydroxyphenylalanine, L-dopa) in a dose of 50 mg/kg and physiological saline (equivalent volume), respectively. Locomotor and emotional activities were studied in the open field test (round field with a diameter of 1.6 m). Animal locomotion was videotaped over 5 min and the data were processed using ImageJ and winTrack software [11]. The rats were then decapitated under light ether narcosis, the brain was fixed in Carnoy

fluid, and paraffin sections were prepared and stained with cresyl violet. The area of profiles of pyramid neurons in the sensorimotor cortex and multipolar neurons of the caudate nucleus was measured and the number of neurons and neuroglial cells in these structures was counted using Leica DMLB microscope and QWin software. The neuroglial index was calculated as the number of neuroglial cells per 1 neuron. The character of interrelationships between various neuronal systems was evaluated by the index of intercellular relations calculated as the ratio of neuroglial indexes (a/b=c) for the corresponding systems: layer V/layer III for intracortical relations; layer V/caudate nucleus and layer III/caudate nucleus for corticosubcortical relations.

Subcellular fractions of the sensorimotor cortex and caudate nucleus were isolated by differential centrifugation [1]. Activity of tyrosine hydroxylase (TH) involved in dopamine synthesis was measured spectrophotometrically [4] at λ=335 with L-tyrosine as the substrate. Activity of monoamine oxidase B involved in dopamine degradation was measured spectrophotometrically [2] at λ=450 with p-nitrophenyl ethylamine as the substrate. Specific activity of the enzymes in both cases was expressed as ΔE_{335}/mg protein per 60 min or ΔE_{450}/mg protein per 60 min, respectively. Protein content in the fractions was measured by the method of Lowry. The data were processed statistically by the Student *t* and Mann—Whitney *U* tests using Statistica 5.0 software.

RESULTS

Control rats freely moved over the entire open field, while experimental animals moved with often and sometimes long-term stops, therefore the length and duration of motion decreased and track curvature increased. Experimental rats more often visited the central zone of the open field and the number of defecation boluses was higher in this group.

In rats receiving L-dopa, the area of neuronal profiles significantly decreased: pyramids of layer III by 8%, small pyramids of layer V by 9%, large pyramids of layer V by 14%, and multipolar neurons of the caudate nucleus by 13%. Variability of the size of neurons, a parameter characterizing deviation from the mean area of nerve cell profile, changed considerably: it decreased by 2.5-3 times for neurons of layer V, increased by 1.5 times for neurons of layer III, and remained unchanged in the caudate nucleus. The neuroglial index in the experimental rats increased by 22% in layer V, tended to decrease by 11% in layer III, and remained unchanged in the caudate nucleus (Table 1). The index of neuroglial relationships in rats of the experimental group increased by 37 and 25% for layer V/layer III and layer V/caudate nucleus ratios, respectively, compared to the corresponding values in the control group and little changed for layer III/caudate nucleus ratio.

Specific activity of TH decreased by 25% in the sensorimotor cortex and tended to decrease by 19% in the caudate nucleus of experimental rats

TABLE 1. Effect of L-Dopa on Neurohistological Characteristics of Rat Brain (*M*±*m*)

Parameter	Control	Experiment	Deviation from control, %
Area of neuronal profile, μ²			
layer III	122.00±2.92	112.00±4.57	-8*
layer V (small pyramids)	142.00±4.19	129.00±1.79	-9*
layer V (large pyramids)	377.00±17.32	324.00±5.64	-14*
caudate nucleus	93.00±2.75	81.00±2.38	-13*
Neuroglial index			
layer III	0.71±0.04	0.63±0.05	-11
layer V	0.87±0.06	1.06±0.07	22*
caudate nucleus	0.75±0.04	0.73±0.05	-3
Index of intercellular (neuroglial) relationships			
layer V/layer III	1.22±0.06	1.70±0.15	39
layer V/caudate nucleus	1.17±0.12	1.45±0.09	24
layer III/caudate nucleus	0.96±0.08	0.86±0.06	-10

Note. Neuroglial index is the number of neuroglial cells per 1 neuron. Index of intracellular relationships characterizes the ratio of neuroglial parameters. Here and in Table 2: minus indicates a decrease in the parameter. *$p<0.05$.

TABLE 2. Effect of L-Dopa on Specific Activity of TH (ΔE_{335}/mg protein/60 min) and Monoamine Oxidase B (ΔE_{450}/mg protein/60 min) in the Brain of Wistar Rats (*M*±*m*)

Enzyme		Control	Experiment	Deviation from control, %
Specific activity of enzymes				
sensorimotor cortex	TH	1.46±0.23	1.10±0.06	-25*
	monoamine oxidase B	0.36±0.05	0.38±0.02	6
caudate nucleus	TH	1.88±0.25	1.53±0.22	-19
	monoamine oxidase B	0.33±0.07	0.44±0.04	33*
Index of neurochemical relationships				
	TH	0.777	0.719	-7
	monoamine oxidase B	1.090	0.863	-21

compared to the control. Monoamine oxidase B activity in the experimental rats increased by 33% in the caudate nucleus and remained practically unchanged in the sensorimotor cortex (Table 2).

Hence, morphofunctional changes in the brain of rats after long-term treatment with L-dopa involved primarily motor system formations, first of all structures of the sensorimotor cortex, which is in line with the observed locomotor disturbances.

Neuroglial relationships play an important role in brain functioning [8,10]. The increase in the neuroglial index observed in layer V, where L-dopa induced most pronounced structural and functional disturbances, attests to the development of compensatory and reparative reaction in the brain under conditions of dopamine system hyperfunction. Changes in cell-cell interaction in the studied structures (ratios of neuroglial indexes for layer V/layer III and layer V/caudate nucleus) demonstrating a shift in the balance of neuroglial relationships under conditions of dopamine system hyperfunction towards projection efferent neurons of layer V are also a manifestation of compensatory and reparative reaction.

Long-term treatment with L-dopa changed activity of enzymes involved into dopamine synthesis (TH) and degradation (monoamine oxidase B). Activity of TH under the effect of L-dopa significantly decreased in the sensorimotor cortex and tended to decrease in the caudate nucleus, while activity of monoamine oxidase B considerably increased in the caudate nucleus and remained practically unchanged in the sensorimotor cortex. Published data suggest that L-dopa excess in the organism disturbs dopamine metabolism [7,9]. Our findings drove us to a conclusion that the observed changes in activities of enzymes involved into dopamine metabolism can be considered not only as a disturbance in dopamine metabolism, but also as a manifestation of compensatory and reparative reactions aimed at the maintenance of brain function.

Thus, modeled hyperfunction of the dopamine system modulates locomotor and emotional activity of experimental animals and changes neurohistological characteristics of cells indicating not only a decrease in structural and functional capacities of cell systems involved into the realization of the locomotor function, but also the appearance of signs of compensatory and reparative reactions. The role of associative neurons of the cortex under conditions of hyperfunction of the dopamine system is preserved and even increases.

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