Dynamics of the Activity of Respiratory Enzymes in Neuronally Isolated Zones of the Neocortex and Symmetrical Regions in Rats

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Activities of the respiratory enzymes succinate dehydrogenase (SDH) and nicotinamide adenyldinucleotide dehydrogenase (NADH-DH) were measured in neuronally isolated neocortical strips and in homotopic regions of the contralateral hemisphere 2, 21, 60, and 90 days after surgery as well as in the cerebral cortex of intact animals. The density of glial cells in the neocortex was evaluated in the same period. Changes in the activities of SDH and NADH-DH in isolated neocortical strips correlated with the dynamics of morphological changes. The data indicate that chronically isolated strips of the neocortex can be an adequate and useful model for electrophysiological, pharmacological, genetic, and other studies.

Key Words: *neocortex; neuronally isolated strip; SDH; NADH-DH; glia*

Cortical zones disconnected from the adjacent regions and subcortical structures with preservation of pial circulation exhibit structural and functional changes which can be observed for several months. Thus, degeneration of afferent terminals is seen 2-3 days after the isolation procedure [3], an increased density of spines and synapses, an increased number of axon collaterals [8], and intensified dendrite sprouting [7] can be observed at the end of the first month. These structural changes result in considerable shifts in the functional state of isolated cortical strip (ICS) [8]. The most informative indices of the tissue conditions are the activities of the main redox enzymes succinate dehydrogenase (SDH) and NADH dehydrogenase (NADH-DH) and their balance [4-6]. In this work we investigated the dynamics of structural changes and energy metabolism in surviving ICS from the rat brain and in homotopic cortical zone of the contralateral hemisphere (HCZ).

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MATERIALS AND METHODS

Experiments were carried out on 60 male Wistar rats weighing 180-200 g. A fragment of the sensorimotor cortex of the left hemisphere was isolated under ketamine (Kalipsol) anesthesia (70 mg/kg) by means of a special laboratory-made instrument. The neocortical activity of the principal energy metabolism enzymes SDH and NADH-DH was determined in 24 experimental and 6 intact rats by a quantitative hystochemical technique using cytospectrophotometry [2]. Enzyme activity in ICS and HCZ was measured 2, 21, 60 and 90 days after surgery. Control indices were measured in the analogous cortical zones of intact animals. Enzyme activity was expressed in arbitrary units (mmol formazan produced by 1 mol protein nitrogen for 1 min at 37°C).

The density of glial cells was determined in ICS and HCZ of the experimental rats (n=24) and in the neocortex of intact rats (n=6). Brain tissue blocks were fixed in 10% formaldehyde in phosphate buffer (pH= 7.2-7.4). Frontal serial sections of 20 μ thickness were

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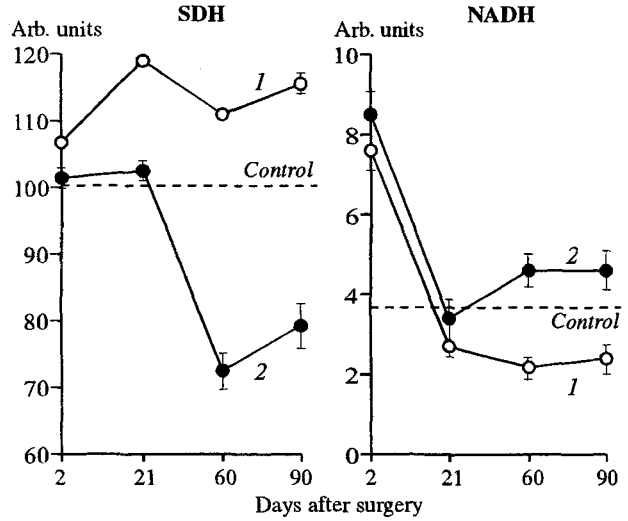

Fig. 1. SDH and NADH activities in neuronally isolated cortical strips (*1*), in homotopic zones of the contralateral hemisphere (*2*), and in the neocortex of intact rat brain at different intervals after surgery.

cut on a cryostat and stained by the method of Nissl. The image analysis software was applied to computerized morphometry of the sections. Glial cell nuclei were counted in 150 random fields of a 1250 μ^2 view area to determine the density of glial cells (10 sections from each rat were analyzed). The data were processed statistically by the Mann—Whitney *U*-test.

RESULTS

The mean value of SDH activity in the sensorimotor cortex of the intact rats (control) was 100.7 arb. units. SDH activity in the left-hemispheric ICS was significantly ($p<0.001$) higher than in the control 2 days after surgery, reaching peak values on the 21th day (Fig. 1). This parameter then decreased but remained above then control level ($p<0.001$) for 2-3 months.

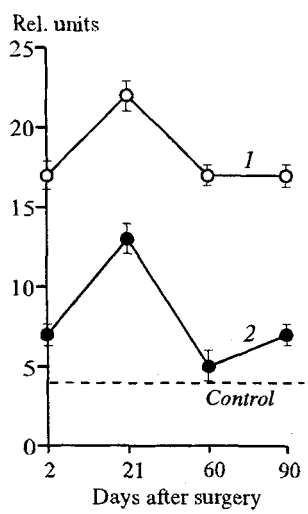

Fig. 2. Glial cell density in neuronally isolated cortical strips (*1*) and homotopic zones of the contralateral hemisphere at different intervals after surgery.

Unexpected data were obtained when measuring the enzymatic activity in the HCZ of experimental rats. Having been slightly higher than in the control 2 days after surgery, the SDH activity continued to increase until day 21, then dropped to 72.5 arb. units after 2 months and remained significantly lower than in the control after 3 months (Fig. 1).

Two days after surgery NADH-DH activity sharply increased in both ICS and HCZ (Fig. 1), in comparison with the control (3.7 arb. units). In ICS it dropped after 21 days below the control ($p<0.001$) and remained at this level for 2 and 3 months. In the contralateral hemisphere the activity of NADH-DH also decreased by the 21th day but then increased and surpassed the control values after 2 and 3 months ($p<0.001$).

Simultaneously with changes in the energy metabolism a dramatic increase in the glial cell density occurred in ICS, which was more pronounced than in HCZ (Fig. 2). The count of glial cells in both ICS and HCZ continued to grow until day 21, it then decreased (2 months) and remained unchanged in ICS and slightly increased in HCZ.

These data show that significant changes in enzyme activity and glial cell density take place both in ICS and HCZ; hence, the contralateral hemisphere cannot be taken as the control.

The postoperative period is characterized by intensive degeneration of neural elements (neurons, axon terminals, and fibers [3]) and by proliferation of glial elements (Fig. 2), which are observed in both ICS and HCZ. The high level of NADH-DH activity on the second day after surgery seems to be necessary to provide the energy for these processes. On the other hand, it can be attributed to the posthypoxic activation occurring on the next day after surgery. At the same time, the dynamics of SDH activity seen in ICS within 21 days is in a good agreement with structural changes occurring within the first month postoperation [8].

Increased activity of SDH and slightly lowered activity of NADH-DH are indicative of an increased excitability of brain tissue [1,4,6]. Our previous studies showed that with increasing brain excitability the level of SDH activity increased by on average 20% compared with the norm [4,6]. In the present study, the increase in SDH activity was observed in ICS after 21 days and later. Therefore, the levels of SDH and NADH-DH activities observed in ICS from day 21 through 90 can be attributed to increased excitability of the nervous tissue. The glial cell density in HCZ dropped to nearly control values after 2-3 months (Fig. 2). The data obtained in other experimental models indicate that the increase in SDH activity is primarily due to its activation in astroglial cells [4]. Therefore, decreased SDH activity in this period can be explained by redistribution of glial cells.

Our results indicate that the ICS preparation represents a viable autonomic system, and the changes in SDH and NADH-DH activities reflect the degeneration/proliferation processes and increased excitability of the nervous tissue. Considerable changes observed in HCZ suggest that the contralateral hemisphere cannot be used as the control in this kind of experiments.

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