Reactivity of Capillaries and Pyramidal Neurons in Rat Cortex under Conditions of Acutely Reduced Circulation

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A study of capillaries and pyramidal neurons of the cortex in white rats subjected to ligation of the common carotid artery reveals that acute reduction of the circulation is characterized by a decreased activity of alkaline phosphatase and Na,K-ATPase as well as a reduced number of capillaries marked with these enzymes, and a lower activity of butyrylcholine esterase. A decreased activity of cytochrome oxidase in neurons suggests the existence of intracellular compensatory-adaptive mechanisms.

Key Words: *reduction of the circulation; capillaries; neurons*

A study of the functioning of the blood-brain barrier (BBB) points to the presence of two opposite mechanisms in its structure: the first allows for the transport to and from the brain, while the second represents barrier systems of the vascular wall [1]. One of the most important transporting mechanisms is Na,K-ATPase effecting the transendothelial transport. Some studies note the role of nonspecific esterase of the cortical vessels in the regulation of the permeability and function of BBB [2,4] in sites where the barrier function is dependent on acetylcholine hydrolysis [5,7].

A number of mechanisms are known to be involved in ischemic damage to the BBB and to the brain; however, there is no unified hypothesis of its development [8].

The changes in barrier and transport activity of the vascular wall and oxidative metabolism of nerve cells in response to acute reduction of the brain circulation were studied by measuring the activity of alkaline phosphatase (AP), Na,K-ATPase, and butyrylcholine esterase (BCE) in capillaries and of cytochrome oxidase (CCO) using histochemical methods.

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MATERIALS AND METHODS

The experiments were carried out on 60 adult unbred white female rats weighing 250-350 g, divided into control and experimental groups. In the experimental animals the common carotid artery was ligated. The control animals were sham-operated without ligation of the vessel. Thirty minutes or 24 hours after the operation the animals were killed and the brain was promptly excised and frozen at 18-20°C. The cryostatic sections were air dried.

Capillaries and pyramidal neurons of layers III and V of the parietal cortex were examined. Microvessels were histochemically contrasted using AP- [5], Na,K-ATPase- [9], and BCE-specific [6] reactions. Localization of CCO in neurons was determined after Nachlass.

The enzymatic activity was evaluated from the optical density of the precipitate with an M85A Vickers densitometer on 20-μ thick sections, prepared from a block with the frozen control and experimental material.

The following metric parameters of the microcirculatory bed were determined: the diameter and length of the capillaries, and the area of the exchange surface. The length of the capillaries was calculated as described by Blinkov and Moiseev

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using the formula for nonuniform distribution of microvessels in the brain tissue. The diameter of the capillaries was measured with an MBI-3 microscope (objective ×40) using an MOV-I-15 ocular micrometer. The results of the morphometric examination were statistically processed using the Student *t* test.

RESULTS

Judging from the final precipitate of the histochemical reactions, the activities of AP and Na,K-ATPase, which are involved in transcapillary exchange, are fairly uniform in the test brain samples of the control animals. There were no differences in metric parameters of the histochemically contrasted capillaries between the hemispheres. BCE more completely stains the vascular bed: the BCE-contrasted capillaries are abundant in the field of view and homogeneously stain brown. The AP- and Na,K-ATPase-contrasted microvessels are fewer than the BCE-contrasted ones. According to current views [2], AP stains the functioning part of the microcirculatory bed.

The AP-specific staining of the microvessels revealed a reduction of the length and exchange surface of capillaries during the first 30 min (Fig. 1) and a decreased activity of the enzyme, both of these increasing over the subsequent 24 h. In both hemispheres a reliable decrease in morphometric parameters, length and exchange surface, was observed. During the first 30 min it was more pronounced in the parietal zone of the right hemisphere. In this period AP activity in the capillary wall in the right hemisphere was reliably higher than in the left (Fig. 1). After 24 hours the intensity and the density of the precipitate in the microvessels of the right hemisphere were higher, whereas the exchange surface was smaller than in the left hemisphere (Fig. 1).

A gradual decrease in both Na,K-ATPase activity and metric parameters of the Na,K-ATPase-contrasted capillaries (reduction of the length, diameter, and the exchange surface) was observed starting from the first 30 min and over 24 h (Fig. 2). The enzyme activity and the exchange surface decreased more in the right parietal zone, while the length of the capillaries decreased more in the left zone (Fig. 2).

A different picture was observed in BCE-contrasted capillaries. Thirty minutes after ligation of the carotid artery the number of microvessels sharply increased in the right and reliably dropped in the left hemisphere (Fig. 3); the exchange surface of the capillaries was considerably reduced (Fig. 3). After 24 h a tendency toward an increase of the exchange surface was revealed, while BCE activity continued to drop, especially in capillaries of the right parietal cortex (Fig. 3).

Thus, acute reduction of the circulation resulted in an increased number of microvessels in the respective hemisphere, probably due to compensatory opening of reserve capillaries. Reduction of the circulation leads to an inhibition of the active transport regulated by capillary AP and Na,K-ATPase and to inactivation of BCE, which is responsible for the barrier function of the vascular wall. The gradual decrease in the activity of the transporting enzymes occurs against the background of a reduced number of functional capillaries.

Activity of CCO, the terminal enzyme of the respiratory chain, may serve as an indicator reflecting the state of oxidative metabolism in the corti-

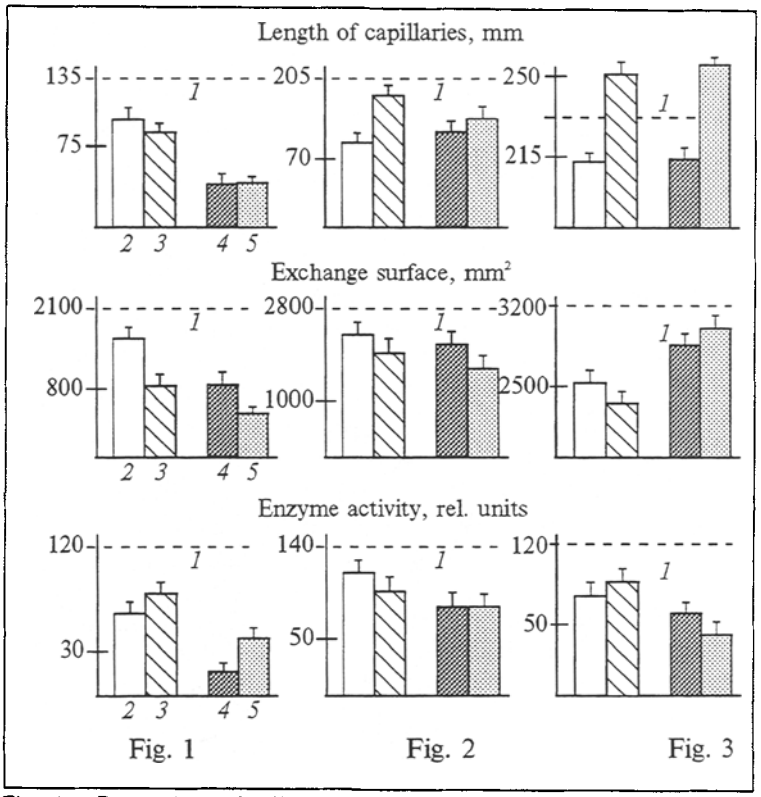

Fig. 1. Dynamics of alkaline phosphatase activity and metric parameters of AP-specific marked capillaries. Here and in Figs. 2–4: *1*) control; *2*) left hemisphere, 30-min reduction; *3*) right hemisphere, 30-min reduction; *4*) left hemisphere, 24-h reduction; *5*) right hemisphere, 24-h reduction.

Fig. 2. Dynamics of Na,K-ATPase activity and metric parameters of ATPase-specific marked capillaries.

Fig. 3. Dynamics of BCE activity and metric parameters of BCE-specific marked capillaries.

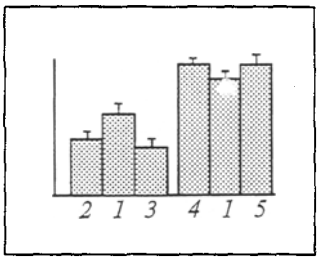

Fig. 4. CCO activity.

cal neurons against the background of inhibition of the barrier and transporting activity of the capillary wall.

The bodies of neurons of intact cortex are sharply outlined. Fine precipitate of diformazane corresponding to the localization of CCO is dispersed over the cytoplasm. There is no reliable difference in enzymatic activity in pyramidal neurons of layers III and V between the right and left hemispheres.

Thirty minutes after reduction of the circulation a decrease of CCO activity was noted (Fig. 4). The density of formazane in the right hemisphere was 62.17%, while in the left hemisphere this decrease was less pronounced (90.17% of the control value). The decreased activity of CCO in response to circulatory disturbances is considered as the first stage in the metabolic rearrangement of the nerve cell [3]. Twenty-four hours later CCO activity in neurons of both hemispheres reliably surpassed the control values (Fig. 4), which probably reflects the enhancement of oxidation-reduction processes in neurons in the course of adaptation to ischemia.

Comparative studies of the activity of barrier and transporting enzymes of capillaries and CCO activity in nerve cells suggest that the latter are relatively autonomous in their reaction to acute reduction of the circulation, which implies the existence of intracellular compensatory and adaptive mechanisms preserving a high level of oxidative metabolism in neurons under conditions of inactivated barrier and transporting systems of the blood-brain barrier.

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