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Dynamics of Postischemic Changes in the Microcirculation in the Rat Cerebral Cortex

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Postischemic changes in the circulation appear primarily in the microcirculatory system. There are few studies of the cerebral microcirculation after ischemia and these are scattered and fragmentary. The aim of the present work was to conduct a complex study of the dynamics of the main parameters of the microcirculation (tissue perfusion with blood, blood saturation, and tissue oxygen consumption) in the cerebral cortex of rats 1 h and 2, 7, 14, and 21 days after ischemia induced by occlusion of both carotid arteries for 12 min with simultaneous controlled hypotension to a level of 45 ± 3 mmHg. The hyperperfusion seen 1 h after ischemia was found to be replaced by degradation of the microcirculation in rat brain tissue lasting the next three weeks, due to suppression of the active mechanisms controlling blood flow and decreases in vessel tone. These features were accompanied by stagnation of the blood, decreases in cortical tissue oxygen consumption, and decreases in blood oxygen saturation.

Keywords: cerebral ischemia, microcirculation, perfusion, saturation.

Questions of the interaction between primary and secondary ischemic damage to the brain have thus far received insufficient study. The outcomes and long-term sequelae of cerebral ischemia are determined both by changes in central hemodynamics and by the state of the blood microcirculation. The microcirculatory system is one of the most important systems in the body, in which postischemic changes are the most apparent [8, 17]. Transient impairments occurring at the level of the microcirculatory bed are often the cause of secondary cerebral ischemia [2]. In the circulatory system, the microcirculatory bed is the connecting component between the arterial and venous vessels and between the blood flow to tissues and its outflow, so the state of the microcirculation depends on a large number of factors acting at various levels, including the tissue level [1]. Impairments to the microcirculation can develop when there are changes to blood influx or efflux or the intensity of oxygen delivery by the blood and the intensity of oxygen consumption by the tissues of the organ. There are currently few studies of the microcirculatory features of the brain after ischemia, and these are scattered and fragmentary in nature [9, 14, 15]. The aim of the present work was to therefore to carry out complex studies of the dynamics of changes in the main parameters of the microcirculation: perfusion of tissue with blood, blood saturation in microvessels, and oxygen consumption by tissues in the cerebral cortex in rats over the 21 days after single episodes of transient cerebral ischemia.

Methods. Experiments were run on Wistar rats weighing 240–320 g (n = 52). Cerebral ischemia was modeled by occlusion of both carotid arteries with controlled hypotension [3]. Postischemic changes were assessed in five separate groups of rats – at 1 h (group I) and at 2, 7, 14, and 21 days (groups II–V) after ischemia – in comparison to values in intact rats. Rats were anesthetized with urethane (i.p., 125 mg/100 g body weight). Mean arterial blood pressure in anesthetized intact rats was 98.2 ± 11.8 mmHg, compared with 99.5 ± 10.9 mmHg in rats subjected to ischemia.

The state of the microcirculation in the cerebral cortex was assessed using an LAKK-M multifunctional laser diagnostic system (LAZMA, Russia), combined with laser Doppler flowmetry (LDF) and optical tissue oximetry (OTO). The LDF method was used to determine statistical measures of the blood microcirculation parameter (MP),

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TABLE 1. Dynamics of Major Microcirculatory Parameters

Parameter	Intact rats, $n = 9$	Values in groups of rats subjected to ischemia				
		1 h, $n = 8$	2 days, $n = 8$	7 days, $n = 9$	14 days, $n = 9$	21 days $n = 9$
MP, perfusion units	16.49 ± 1.69	26.67 ± 4.58*	15.78 ± 3.4	18.2 ± 3.07	24.15 ± 3.96*	21.52 ± 1.72*
MAO, perfusion units	1.93 ± 0.38	1.4 ± 0.35	1.08 ± 0.24*	1.2 ± 0.25*	1.16 ± 0.16*	1.83 ± 0.32
CV, %	8.45 ± 2.07	6.12 ± 1.26	5.92 ± 1.49	5.73 ± 0.52*	6.54 ± 1.79	8.36 ± 1.03

Here and in Table 2: *$p < 0.05$ compared with intact rats.

TABLE 2. Tissue Oximetry in Intact Rats and Rats Subjected to Ischemia

Parameter	Intact rats, $n = 9$	Time after ischemia				
		1 h, $n = 8$	2 days, $n = 8$	7 days, $n = 9$	14 days, $n = 9$	21 days $n = 9$
Sm	5.03 ± 0.65	4.58 ± 0.65	5.43 ± 0.94	4.73 ± 0.55	4.89 ± 1.02	3.74 ± 0.4*
SO_2, %	85.23 ± 4.47	88.73 ± 3.81	84.18 ± 5.52	87.83 ± 3.81	79.51 ± 5.56	75.13 ± 4.81*
Vr, %	17.72 ± 2.03	18.18 ± 1.58	19.01 ± 3.11	13.73 ± 3.91	22.54 ± 2.7*	22.03 ± 1.74*

i.e., changes in blood flow (tissue perfusion with blood) per unit time in a tissue volume of about 1 mm^3, arithmetic mean perfusion (M), mean square deviation of the amplitude of blood flow oscillations (MAO), and the coefficient of variation (CV), which shows the relationship between tissue perfusion with blood and the extent of variability in this parameter and characterizes microvascular vasomotor activity. The OTO method assesses the mean relative level of blood saturation in the microcirculatory bed (SO_2) and the volumetric tissue blood filling (Vr). The resulting values were used to calculate indexes determining the interaction between perfusion and saturation [1]: the index of relative perfusional oxygen saturation in the microcirculation (Sm) and specific tissue oxygen consumption (U):

$$Sm = SO_2/M,$$

$$U = (100 - SO_2)/Vr.$$

The probe of the instrument was placed in a window ($S \approx 2$ cm^2) window drilled into the dorsal surface of the skull with removal of the dura mater in the window. In each rat, measurements were made at six points located in the frontal, parietal, and occipital areas of both hemispheres of the cerebral cortex. Trace duration at each point was 12 min.

Distributions of results were tested for normality and processed by variational statistics and presented as arithmetic mean values and mean errors; significant differences were identified using Student's test.

Results. The data obtained here show that the arithmetic mean measure of the microcirculation parameter (MP) in intact rats was 16.49 ± 1.69 perfusion units; MP increased 1 h after ischemia by 61.83% from the value in intact rats ($p < 0.05$) (Table 1).

Mean values of the microcirculation parameter in groups of rats on postischemia days 2 and 7 were not significantly different from values in intact rats. Then, on postischemia days 14 and 21, MP again increased, by a mean 38.3% ($p < 0.05$) (Table 1). Increases in MP were seen only in the parietal areas of the cerebral hemispheres (Fig. 1).

Thus, our studies established that the rat cerebral cortex showed nonuniform changes in the mean level of perfusion over the 21 days after ischemia. However, some investigators take the view [12] that mean MP values are not always informative but can vary over wide ranges. The mean square deviation of the amplitude of oscillations in blood flow (MAO), characterizing changes over time, is more informative. Significant decreases in MAO were seen from day 2 to day 14 after restoration of blood flow (Table 1). By postischemia day 7, this led to a decrease in the coefficient of variation (CV), which characterizes the relationship between tissue perfusion and the extent of its variability (Table 1).

The degradation of tissue microcirculation seen here in rats with ischemia was also accompanied by a decrease in cerebral cortex tissue oxygen consumption for 14 days after disease. Specific tissue oxygen consumption (U) decreased by a mean of 64.6% ($p < 0.05$) (Fig. 2).

At 21 days after ischemia, on the background of an increase in cerebral cortex tissue perfusion, a reduction in blood saturation was seen in the microcirculatory bed: blood saturation SO_2 decreased by a mean 11.7% ($p < 0.05$) (Table 2), while the index of perfusional oxygen saturation Sm decreased by a mean of 25.8% ($p < 0.05$).

During the period from postischemia day 14 to day 21, changes in the microcirculation occurred on the background of an increase in volumic blood filling (Vr) by a mean of 27.2% ($p < 0.05$) (Table 2).

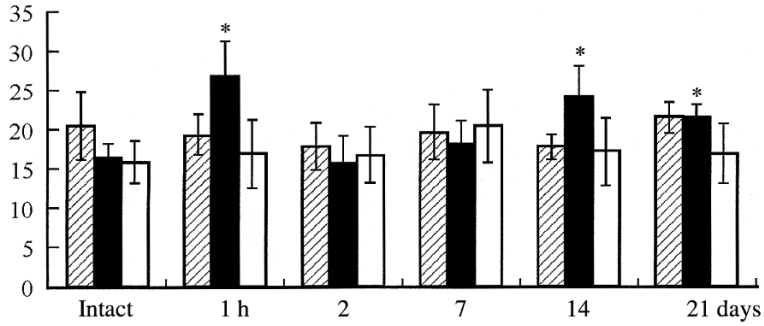

Fig. 1. Microcirculation in areas of the cerebral hemispheres in rats. The ordinate shows the measure of microcirculation, perfusion units; the abscissa shows groups of rats (intact and at different postischemia time points). Shaded columns show the frontal lobe; dark columns show the parietal lobe; light columns show the occipital lobe. *$p < 0.05$ compared with the corresponding values in intact rats.

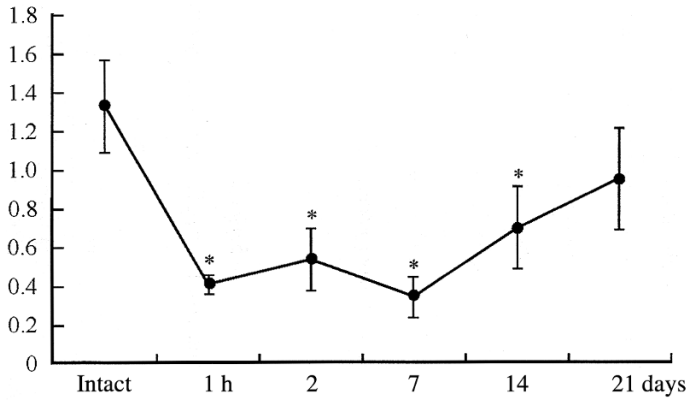

Fig. 2. Changes in the index of specific tissue oxygen consumption. The ordinate shows specific tissue oxygen consumption U; the abscissa shows groups of rats (intact and at different postischemia time points). *$p < 0.05$.

Discussion. The results obtained here provide evidence that single episodes of transient cerebral ischemia lead to changes in mean cerebral cortex perfusion in rats. An increase in perfusion was seen 1 h after ischemia, possibly associated with the occurrence of postischemia hyperemia, the so-called "luxury perfusion" arising as a result of abundant influx of blood via collaterals, the release of vasoactive and anti-inflammatory metabolites from ischemic tissue, and changes in the rheological properties of the blood [11, 13, 16]. Over the subsequent seven days after ischemia, the mean value of the microcirculation parameter in rats subjected to ischemia was not significantly different from values in intact animals. A renewed increase in MP was seen only 14 days after ischemia and MP then remained elevated compared to the value in intact animals on postischemia day 21. It is important to note that the increase in MP in rats subjected to ischemia was seen only in the parietal area of the cerebral hemispheres. It may be that the absence of any significant increase in MP in the occipital and frontal areas of the brain during the postischemic time period should be regarded not as normal, but as a sign of ischemic reperfusion injury to brain microvessels, leading to inadequate perfusion. As we have demonstrated previously [3], these areas of the cortex show a significant reduction in the blood flow rate during the first days after transient ischemia. This can lead to the formation of erythrocyte stasis and sludge, followed by thrombus formation in vessels, such that restoration of blood flow in these areas can be accompanied by incomplete reperfusion of previously ischemic tissue [4].

From postischemia day 2, the mean square deviation of the amplitude of blood flow oscillations characterizing its variability over time also changed. During the period from postischemia day 2 to day 7, the perfusion parameter remained unaltered but MAO decreased, pointing to a reduction in the modulation of microcirculatory blood flow due to suppression of the active mechanisms regulating the microcirculation (endothelial secretion and the vasomotor mechanism of regulation of the microcirculation) and decreases in vascular tone [6, 12]. By postischemia day 7, there was also a reduction in the coefficient of variation (CV), characterizing the relationship between tissue perfusion and its level of variability (Table 1), which is taken to reflect a general degradation of the microcirculation [1, 6, 7]. At 14 days after ischemia, the amplitude of blood flow variations decreased on the background of an increase in PM. The microcirculation parameter provides evidence of

altered perfusion and characterizes the mean flow of erythrocytes per unit tissue volume in the area being probed in the time period recorded. On the one hand, the increase in this parameter with a simultaneous reduction in MAO may be linked with weakening of arteriolar vascular one, which leads to an increase in the volume of blood in the arterioles [1]; on the other hand, the value is proportional to the number of erythrocytes [1, 5]. Volumic blood filling (Vr), reflecting the concentration of erythrocytes in the volume of blood being probed, increased significantly by postischemia day 14 and remained elevated on postischemia day 21 (Table 2). Thus, the increase in MP may be linked with signs of blood stagnation in the microcirculatory component of the vascular network of the cerebral cortex [1, 5].

The degradation of the tissue microcirculation seen in these experiments in rats subjected to ischemia in the first 14 days after ischemia was also accompanied by a decrease in cerebral cortex tissue oxygen consumption (Figs. 1 and 2), which is evidence for the lack of effective oxygenation throughout the postischemic period. On postischemia day 21, there was a reduction in blood oxygen saturation on the background of an increase in perfusion and an increase in volumic blood filling in the microcirculatory bed of the cortex, as indicated by a decrease in blood saturation and the index of perfusional oxygen saturation [10]. Impairments to the balance between the rate of delivery and oxygen consumption in brain tissue leading to decreases in blood oxygen content constitute an adverse factor and can increase the likelihood of secondary cerebral ischemia.

Thus, our studies show that despite excessive perfusion in the first hour after ischemia, a single episode of transient cerebral ischemia leads overall to degradation of the microcirculation in cerebral cortex tissue persisting to postischemic day 21 in rats due to suppression of the active mechanisms regulating blood flow and decreases in vessel tone. Suppression of the regulation of blood flow is accompanied by blood stagnation and decreases in effective tissue oxygenation and may ultimately lead to repeated cerebral ischemia.

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