GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effect of Emotional Stress on Hemoglobin Oxygen Affinity in Low Resistant Animals under Normal Conditions and During Cerebral Ischemia

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Hemoglobin oxygen affinity within the estimated physiological range plays an adaptive antioxidant role during acute cerebral ischemia. This range depends on individual emotional resistance. Brain ischemia induced by common carotid artery occlusion in low resistant Wistar rats increased hemoglobin oxygen affinity by 12% during the acute period. Emotional stress also increased hemoglobin oxygen affinity and determined shifts in this parameter during the development of cerebral ischemia: moderate increase in hemoglobin oxygen affinity (<25%) was followed by further increase in this parameter, while more pronounced shift (>25%) resulted in a significant drop in this parameter due to hemoglobin deoxygenation. Adaptation to stress shifted the upper physiological limit for self-regulation of this process.

Key Words: *hemoglobin oxygen affinity; cerebral ischemia; emotional resistance; emotional stress*

Disturbances in oxygen metabolism in the brain caused by circulatory disorders activate processes leading to generation of reactive oxygen species. These changes are followed by free radical damage to cells. Emotional stress (ES) also activates lipid peroxidation (LPO) [1,8]. In animals predisposed to stress and most sensitive to cerebral ischemia, 18-h ES increased the content of LPO products in brain structures more significantly compared to resistant specimens (by 2 times) [1]. Cerebral ischemia developing against the background of ES is characterized by more severe course, is associated with progressive decrease in cerebral blood flow and death of 90% animals. Since a negative correlation was found between LPO intensity and hemoglobin oxygen affinity [4], it was hypothesized that the antioxidant status depends on hemoglobin oxygen affinity [5]. Changes in oxygen transport function of erythrocytes are the major mechanism of adaptation to circulatory disorders [2,10], therefore it was interesting to study the effect of ES on hemoglobin oxygen affinity in stress-predisposed animals under normal conditions and during cerebral ischemia.

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MATERIALS AND METHODS

Experiments were performed on 27 male Wistar rats weighing 350-400 g and characterized by low resistantce to ES (by locomotor activity in the open-field test [6]. Previous studies showed that Wistar rats exhibiting passive behavior in the open field are predisposed to ES and highly sensitive to cerebral ischemia [1]. The animals were divided into 3 groups. Group 1 included intact rats (n=8). Group

2 animals (n=10) were exposed to aggressive conflict situation (fixation by tail to the wall of the cage) for 18 h before the start of the study [9]. Group 3 rats (n=9) were subjected to 4 stress exposures with a 1-week interval: transport from the Stolbovaya nursery and housing in novel vivarium conditions; maintenance at 0°C for 5 days; maintenance at 5°C for 3 days; and aggressive conflict situation. Group 3 included the animals that did not have respiratory and lung diseases 1 week after 2-fold exposure to cold conditions. Cerebral ischemia in animals was produced by bilateral occlusion of the common carotid arteries (CCAO) under nembutal anesthesia (45 mg/kg). Blood samples (0.5 ml) were taken from the femoral artery and stabilized with heparin. The procedure was performed before and 1 h after CCAO. The blood (10 μl) was injected into a photometric temperature-controlled (37°C) sealed cell with complete light absorption. This cell contained 18 ml isotonic phosphate buffer (Sigma diagnostics, pH 7.6, 25°C) and was equipped with a stirrer (350 rpm) and oxygen sensor (LP7 polarograph, PRAHA). O_2 was replaced with nitrogen. After depressurization of the cuvette, the steady-state dynamics of hemoglobin oxygenation was studied during slow saturation of the medium with oxygen at λ=420 nm. Hemoglobin oxygen affinity was estimated from Po_2, at which the degree of hemoglobin saturation corresponded to 50% (P_{50}). The results were analyzed by paired and unpaired Student's t test.

RESULTS

Under normal conditions, P_{50} in anesthetized male Wistar rats with low resistance to ES and cerebral ischemia was 28.83±2.10 mm Hg. This value of P_{50} reflects a normal level of oxygenation and O_2 release from hemoglobin [2,12]. Our results are consistent with published data on rats with low resistance to hypobaric hypoxia [7]. P_{50} decreased by 3.47±2.91 mm Hg after CCAO, which was related to an increase in hemoglobin O_2 affinity (Fig. 1). Since tissue demands are normally satisfied by O_2 released during dissociation of 30-35% oxyhemoglobin [2], the decrease in P_{50} by 4 mm Hg after CCAO reflects a significant increase in hemoglobin O_2 affinity, which improves blood oxygenation in the lungs (complete saturation) even at reduced alveolar Po_2, but decreases O_2 release in tissues by 20-30%, which is considered as a possible cause of hypoxia in tissues [12]. The content of LPO products (*e.g.*, malonic dialdehyde) in brain tissue of low-resistant animals is usually higher than in high-resistant animals [1]. Changes in blood supply to the brain after CCAO initiates cascade biochemical reactions, which are followed by more pronounced LPO activation. The observed significant increase in hemoglobin O_2 affinity is probably directed toward reduction of O_2 supply to brain tissue. These changes contribute to a decrease in the formation of LPO products and provide O_2 utilization to meet tissue demands in the absence of reactive increase in neuronal activity observed in stress-predisposed animals immediately after CCAO [1]. The increase in hemoglobin O_2 affinity after CCAO and decrease in cerebral blood flow may increase the severity of cerebral hypoxia and contribute to dysregulation of oxygenation and deoxygenation of hemoglobin accompanied by a sharp decrease in hemoglobin O_2 affinity. This, together with higher intensity of free radical processes under normal conditions, leads to the development of structural damage to biological membranes and decrease in activity of the antioxidant system, which determines high mortality (40%) of low resistant specimens from cerebral ischemia [1].

Long-term aggressive conflict situation causes ES accompanied by massive release of catecholamines into the blood [1,8]. These hormones increase O_2 consumption in tissues [3]. Hemoglobin O_2 affinity in group 2 animals significantly increased after ES (p<0.05). This conclusion was derived from the decrease in P_{50} by 7.29±1.61 mm Hg (Fig. 1). The increase in hemoglobin O_2 affinity determined further changes in this parameter during the acute period of cerebral ischemia. When hemoglobin O_2 affinity increased by 17% (3 animals, ΔP_{50}= 4.75±1.90 mm Hg, p<0.05), CCAO was followed by further increase in this parameter. These

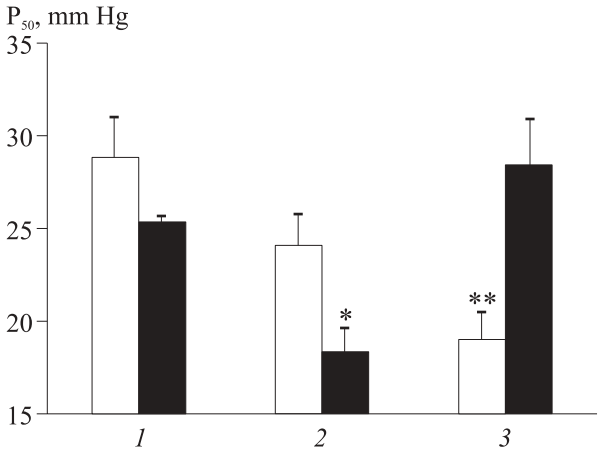

Fig. 1. Variations in Po_2 at which hemoglobin was saturated by 50% (P_{50}) under conditions of cerebral ischemia after ES. Light bars, before ischemia; dark bars, ischemia. Normal (*1*); 17% decrease in P_{50} after ES (n=*3*, *2*); and 34% decrease in P_{50} after ES (n=*6*, *3*). *p<0.02, **p<0.001 compared to normal.

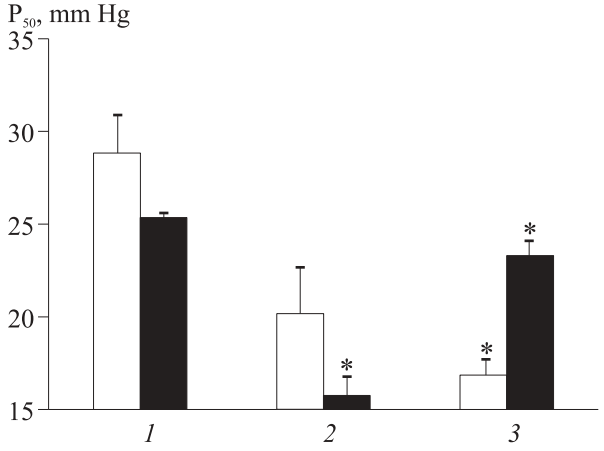

Fig. 2. Variations in Po_2 at which hemoglobin was saturated by 50% (P_{50}) under conditions of cerebral ischemia after repeated stress. Light bars, before ischemia; dark bars, ischemia. Normal (*1*); 17% decrease in P_{50} after repeated stress (*n*=*2*, *2*); and 34% decrease in P_{50} after repeated stress (*n*=*6*, *3*). **p*<0.001 compared to normal.

changes probably played a regulatory role and were directed towards the decrease in O_2 supply to brain tissue. Previous studies showed that even 2-h conflict situation is accompanied by LPO activation. The degree of LPO increased more significantly during stress of a longer duration [1]. In 6 animals exhibiting a 2-fold greater increase in hemoglobin O_2 affinity (34%, ΔP_{50}=9.82±1.81 mm Hg, p<0.001), CCAO was followed by a 50% decrease in this parameter. Hemoglobin O_2 affinity probably exceeded its upper physiological limit after the combined action of ES and CCAO and subsequent dysregulation of oxygenation and deoxygenation of hemoglobin. One rat demonstrating a poststress increase in P_{50} to 37 mm Hg (28% decrease in hemoglobin O_2 affinity) died 30 min after CCAO. It was probably related to dysregulation. Published data show that the rise in P_{50} even by 2.0-4.2 mm Hg is equivalent to a 20-30% increase in oxygen release in tissues due to the decrease in hemoglobin O_2 affinity. It can be hypothesized that the decrease in hemoglobin O_2 affinity after ES alone or in combination with CCAO increases oxyhemoglobin dissociation and O_2 supply to brain tissue during depletion of endogenous antioxidants. These changes contribute to activation of LPO, which was revealed in brain tissue of stress-predisposed animals under similar conditions [1]. Dysregulation of oxygen transport in the blood is probably one of the pathogenetic mechanisms responsible for 90% mortality rate of animals from cerebral ischemia after ES.

The stress response is directed towards an increase in the resistance to extreme conditions and development of adaptation at the expense of activation of the genetic apparatus and changes in cell metabolism. Group 3 animals were characterized by various changes in hemoglobin O_2 affinity 1 week after repeated stress: an increase by 30% (*n*=2) and 41% (*n*=6, p<0.001) or in one case a decrease in hemoglobin O_2 affinity by 11% (judging from P_{50} change, Fig. 2). CCAO was followed by a decrease (by 4.41±1.79 mm Hg) or increase in P_{50} (by 6.90± 0.76 mm Hg, p<0.001) or animal death, respectively. The observed changes were similar to those typical of stressed animals. However, these changes occurred at lower P_{50} (p<0.05) and higher hemoglobin O_2 affinity. The increase in the upper physiological limit of hemoglobin O_2 affinity can be considered as an adaptive response to metabolic changes in cells and tissues of vital organs after repeated stress and rest period.

We evaluated the physiological range within which hemoglobin O_2 affinity plays an adaptive antioxidant role during acute cerebral ischemia. The limits of this range depend on individual emotional resistance. Exceeding the upper limit of this range is a pathogenetic factor for complications after emotional stress or other stress that result in death from cerebral ischemia. The decrease below the lower limit probably explains the failure of pharmacotherapy for oxygen deficiency (*e.g.*, during local tissue ischemia).

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