ENZYME ACTIVITY AND CONCENTRATION OF METABOLITES OF THE GLYCEROPHOSPHATE SHUTTLE CYCLE IN THE CORTEX AND MEDULLA DURING HYPERBARIC OXYGEN THERAPY FOR ACUTE BLOOD LOSS

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KEY WORDS: hyperbaric oxygenation; acute blood loss; sensomotor cortex; medulla; glycerophosphate shuttle cycle.

The study of the mechanisms of action of hyperbaric oxygenation (HBO) is not only of general pathological interest, but also of applied clinical importance because oxygen, under increased pressure, is a pharmacological agent which determines the therapeutic effect in many diseases [1, 5]. Among the oxygen-dependent systems, an important role in HBO is played by the redox system, which is responsible for energy formation in mitochondria. Effective tissue respiration requires a continuous supply of cytoplasmic hydrogen to the respiratory chain of mitochondria, in the composition of metabolites of shuttle cycles, the enzymes of which take part in the coordination of respiration and glycolysis [6, 11]. The glycerophosphate shuttle mechanism [13] is of great importance for function of the nervous system. Meanwhile, the dynamics of its metabolic reactions in the hypoxic brain against the background of HBO awaits elucidation.

In the investigation described below activity of mitochondrial and cytosol glycerophosphate dehydrogenase and the concentrations of reduced and oxidized NAD and of glycerol-3-phosphate (GP) in the brain were studied during hyperbaric oxygen therapy for acute blood loss.

EXPERIMENTAL METHOD

Six series of experiments were carried out on cats of both sexes (n = 101), weighing 3.20 ± 0.07 kg and anesthetized with thiopental-sodium (20 mg/kg, intravenously). The investigations were carried out before blood loss (series I, control), at various stages of the posthemorrhagic period: compensation (10th and 70th minutes, series II and III respectively) and decompensation (series IV), and after HBO on anemic (series V) and healthy (series VI) animals. Acute blood loss was produced by fractional bleeding from the femoral artery in volume of 24.0 ± 0.8 ml/kg body weight, as a result of which the blood pressure (BP), recorded by a mercury manometer in the femoral artery, fell to 8.0 ± 0.2 kPa. The animals were given HBO with medical oxygen in a pressure chamber under a pressure of 303.9 kPa for 60 min.

Concentrations of GP and NAD were studied by enzymic methods of analysis [8] in tissues of the sensomotor cortex and medulla, frozen with liquid nitrogen. The concentration of the reduced form of NAD (NADH) was determined by a fluorometric enzyme method [14]. Activity of flavin-adenine dinucleotide-dependent glycerol-3-phosphate dehydrogenase in the mitochondria (MGPD) isolated by the method in [4], was studied spectrophotometrically, using phenazine methosulfate and dichlorophenolindophenol (DCPIP) as electron acceptor [12]. Activity of cytosol NAD-dependent glycerol-3-phosphate dehydrogenase (CGPD) was determined spectrophotometrically by the UV test [10] in supernatant obtained after centrifugation at 45,000g for 90 min. The protein concentration in the subcellular fractions was determined by a modified Lowry's method [9].

The experimental results were subjected to statistical analysis by Student's parametric t test [2].

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TABLE 1. GP Concentration and MGPD and CGPD Activity in Cat Brain during Acute Blood Loss and HBO (M ± m)

Series of experiments	Sensomotor cortex			Medulla		
	GP	MGPD	CGPD	GP	MGPD	CGPD
I. Control (8/8)	0,169±0,014	0,888±0,066	0,124±0,019	0,172±0,022	0,683±0,054	0,227±0,017
II. Compensation, 10th minute (8/9)	0,170±0,013	0,890±0,074	0,125±0,010	0,204±0,029	0,730±0,078	0,225±0,018
III. Compensation, 70th minute (8/9)	0,174±0,016	0,889±0,124	0,099±0,008	0,159±0,011	0,808±0,139	0,187±0,034
IV. Decompensation, 60 ± 14 minute (8/9)	0,296±0,040 $P_{I-III}<0,05$	0,832±0,069	0,058±0,011 $P_{I-III}<0,01$	0,269±0,024 $P_{I-III}<0,01$	0,724±0,054	0,124±0,027 $P_{I-III}<0,01$
V. HBO (8/10)	0,172±0,011 $P_{IV}=0,01$	1,175±0,092 $P_{I, II, III}<0,05$	0,112±0,006 $P_{IV}<0,001$	0,185±0,016 $P_{IV}<0,05$	0,909±0,054 $P_{I, IV}<0,05$	0,233±0,022 $P_{IV}<0,01$
VI. HBO to healthy animals (8/8)	0,197±0,015	0,825±0,050	0,115±0,017	0,191±0,013	0,751±0,044	0,198±0,037

Legend. P_I) Significance of differences between parameters compared with experiments of series I, P_{II}) with series II, P_{III}) with series III, P_{IV}) with series IV. GP concentration given in millimoles/kg wet weight of tissue, MGPD activity in micromoles DCPIP/mg protein/sec, and CGPD activity in nanomoles NADH/mg protein/sec. Number of animals given in parentheses. Numerator) for GP, denominator) for MGPD + CGPD.

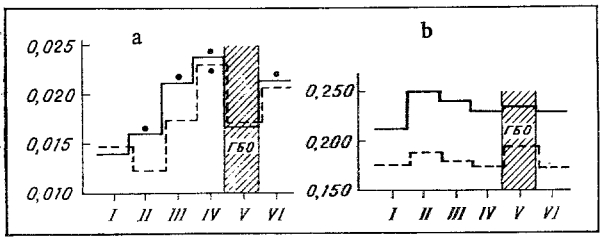

Fig. 1. Concentrations of NADH (a) and NAD (b) in brain tissue of cats during acute blood loss and hyperbaric oxygenation. Abscissa, series of experiments; ordinate, value of parameter (in mmoles/kg wet weight of tissue). Continuous line represents sensomotor cortex; broken line, medulla. Dots indicate P < 0.05 compared with control.

EXPERIMENTAL RESULTS

The NADH concentration in the sensomotor cortex of animals in the compensated stage of blood loss (BP = 8.0 ± 0.2 kPa) after 10 min (series II) and 70 min (series III) of posthemorrhagic hypotension was 22 and 54% higher respectively while the initial NADH concentration was preserved in the medulla (Fig. 1). In the stage of decompensation (BP = 1.3 ± 0.3 kPa, agony), which developed in 56% of animals untreated with oxygen, 60 ± 14 min after blood loss (series IV) the NADH concentration was increased both in the cortex (by 75%) and in the medulla (by 65%). The progressive rise of the NADH level with an increase in the severity of blood loss is evidence of depression of oxidative processes, due in these animals to a decrease in the supply of oxygen to the brain and its partial pressure in nerve tissues [7].

In the decompensated stage of blood loss there was a marked increase in the GP concentration in the cortex by 75% and in the medulla by 56% (Table 1), which can be explained by blocking of its conversion into dihydroxyacetone phosphate at the MGPD level, because of the dramatic deficiency of oxygen as electron acceptor in the respiratory chain. Depression of CGPD activity in the cortex by 53% and in the medulla by 45%, reflecting disturbance of the function of the glycerophosphate shuttle mechanism, may have a significant effect on lactate production, for the brain lactate concentration was increased threefold under these conditions [7]. The depressed activity of CGPD, which has high affinity for NADH and, under normal conditions, inhibits glycolysis [11], in the presence of hypoxia and against a background of a raised NADH level, creates optimal conditions for interaction with lactate dehydrogenase, reducing pyruvate into lactate.

During HBO therapy of the anemic animals, which was used in the initial stage of compensation, GP rose to 10.0 ± 0.9 kPa. After decompression (series V) the NADH and GP levels

and CGPD activity in the cortex and medulla, unlike these parameters in animals not treated with oxygen, did not differ significantly from the control. Meanwhile MGPD activity was increased by 32% in the cortex and by 33% in the medulla. Activation of MGPD by oxygen, enabling the transfer of electrons through dehydrogenation of GP to the mitochondrial respiratory chain, is evidence of intensification of the mitochondrial part of the glycerophosphate shuttle mechanism. Evidence of the effective operation of the mitochondrial respiratory chain under these conditions is given by preservation of the normal ATP level in the anemic brain of oxygenated animals [3]. Meanwhile prevention by HBO of inhibition of CGPD, taking its competitive relations with lactate dehydrogenase for NADH into account [11], may be regarded as one mechanism utilizing the lactate level in the anemic brain under hyperoxic conditions [7].

In healthy animals HBO (BP = 21.3 ± 0.3 kPa) caused no changes in the GP and NAD concentrations or MGPD and CGPD activity in the brain (series VI). However, the NADH level in the sensomotor cortex was raised by 56%; this probably reflects the period of cerebral hypoxia arising after decompression due to the reduction of the cerebral blood flow during HBO [1, 5].

HBO after acute blood loss thus activated MGPD, the enzyme responsible for GP dehydration and for the supplying of cytoplasmic hydrogen to the mitochondrial respiratory chain, in the sensomotor cortex and medulla, and prevented elevation of the GP level, inactivation of CGPD, and an increase in the NADH concentration, limiting lactate production. Under conditions of hyperbaric oxygen therapy, reactions of the glycerophosphate shuttle cycle in the anemic brain are directed toward reducing the degree of development of typical pathophysiological processes, such as bioenergetic insufficiency and lactate acidosis.

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