ENERGY METABOLISM IN THE CORTEX AND DIENCEPHALON DURING HYPERBARIC OXYGEN THERAPY OF ACUTE BLOOD LOSS

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alon.

Energy metabolism has been found to be highly sensitive to a raised pressure of oxygen, which can considerably reduce the formation of ATP molecules [8, 13]. The response of the brain in such cases may be an early warning of exposure to the action of oxygen [10]. However, there is evidence that during hyperbaric oxygenation (HBO) the ATP content in the brain rises and the bioenergetic potential of nerve cells increases if previously they had been under hypoxic conditions [4]. However, the role of the most important processes of energy metabolism in the mechanisms of the therapeutic action of HBO in many pathological states, including acute blood loss, is still not clear.

The aim of this investigation was to study activity of some enzymes of energy metabolism and the concentrations of adenine nucleotides (ATP, ADP, AMP) and creatine phosphate (CP) in the brain (cortex, thalamus, hypothalamus) during hyperbaric oxygen therapy of acute blood loss.

EXPERIMENTAL METHOD

Altogether six series of experiments were carried out on 134 short-haired gray cats of both sexes weighing 3.2 kg, anesthetized with thiopental (20 mg/kg, intravenously).

Substrate concentrations and enzyme activity were studied in the animals before bleeding (series I), at different stages of the posthemorrhagic period — compensation (series II and III) and decompensation (series IV), and after HBO of the anemic (series V) and healthy (series VI) animals. Acute blood loss was brought about by repeated withdrawal of 24.0 ± 0.8 ml/kg blood from the femoral artery, until the blood pressure (BP) recorded by a mercury manometer fell to 80/60 gPa (60/50 mm Hg). The animals were subjected to HBO in a pressure chamber with medical oxygen under a pressure of 3039 gPa (3 atm) for 60 min.

The partial pressure of oxygen (pO_2) in the sensomotor cortex and hypothalamus was measured polarographically [1] by a stereotaxic method. Enzymic methods were used [11] to study concentrations of ATP, ADP, AMP, and CP in tissue of the sensomotor cortex and subcortex (thalamus + hypothalamus), frozen in liquid nitrogen. The energy charge (EC) was calculated:

$$EC = \frac{ATP + 0.5\ ADP}{ATP + ADP + AMP}.$$

Creatine kinase activity was determined spectrophotometrically by reduction of NADP [11] in mitochondria isolated by the method in [7], and in cytosol obtained after centrifugation at 45,000g for 90 min. Activity of Mg-ATPase in the mitochondria was determined from the removal of inorganic phosphate [5]. Mg-ATPase activity in medium with the addition of Na^+ (100 mM) and K^+ (20 mM) ions was independent of the presence or absence of ouabain, evidence of a high degree of purification of the mitochondria from Na,K-ATPase of the microsomal fraction. Cytochrome oxidase (CCO) activity in the mitochondria was determined spectrophotometrically [14]. Protein in the subcellular fractions was determined by a modified Lowry's method [12].

The experimental results were subjected to statistical analysis by Student's parametric test [3] and Wilcoxon's nonparametric T test [2].

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TABLE 1. Mg-ATPase Activity (in nmoles P_i/mg protein/sec), Adenine Nucleotide Content (in mmoles/kg tissue), and EC in the Brain during Acute Blood Loss and HBO (M ± m)

Part of brain	Series of experiments	Experimental conditions		Mg-ATPase	ATP	ADP	AMP	EC
Sensomotor cortex	I	Control	(9/10)	2,36±0,15	2,36±0,15	0,70±0,05	0,21±0,02	0.83±0,01
		Blood loss: compensation						
	II	10th minute	(8/8)	2,77±0,11*	2,41±0,10	0,74±0,05	0,26±0,03	0,81±0,01
	III	70th minute	(8/8)	2,77±0,36	2,09±0,13	0,71±0,04	0,31±0,03*	0,78±0,01*
	IV	decompensation	(8/11)	2,64±0,29	1,95±0,16*	0,85±0,06	0,44±0,07*	0,72±0,02*
	V	HBO	(8/10)	2,67±0,13	2,50±0,20	0,78±0,06	0,33±0,03†	0.80±0,02
	VI	HBO of healthy animals	(7/9)	2,44±0,21	2,22±0,15	0,75±0,07	0,31±0,03*	0,80±0,02
Diencephalon	I	Control	(9/10)	2,74±0,14	1,97±0,14	0,63±0,05	0,23±0,02	0,80±0,01
		Blood loss: compensation						
	II	10th minute	(8/8)	3,29±0,18*	2,31±0,19	0,74±0,05	0,35±0,06	0,78±0,02
	III	70th minute	(8/8)	3,38±0,27*	1,98±0,16	0,75±0,06	0,34±0,03†	0,76±0,02
	IV	decompensation	(8/11)	3,14±0,27	1,42±0,14*	0,82±0,04†	0,49±0,05†	0,67±0,02†
	V	HBO	(8/10)	3,41±0,29*	2,28±0,17	0,79±0,04*	0,36±0,04*	0,78±0,02
	VI	HBO of healthy animals	(7/9)	2,84±0,26	2,01±0,15	0,69±0,05	0,32±0,04*	0,78±0,02

Legend. Number of animals shown in parentheses (numerator — for ATPase, denominator — ATP, ADP, AMP, and EC). *$P < 0.05$, †$P < 0.01$.

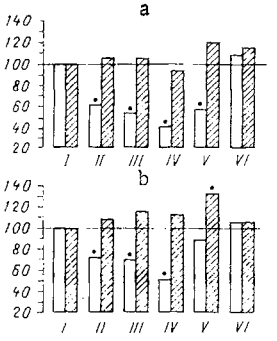

Fig. 1. CCO activity (shaded columns) and pO_2 (unshaded columns) in cat brain tissue after acute blood loss and HBO: a) sensomotor cortex, b) diencephalon. Dots above columns indicate $P < 0.05$ compared with control. Abscissa, series of experiments: I) control, II) stage of compensation (10th minute), III) stage of compensation (70th minute), IV) stage of decompensation, V) blood loss + HBO, VI) HBO of healthy animals; ordinate, parameter studied (in %).

EXPERIMENTAL RESULTS

The stage of compensation of acute blood loss (series II, BP = 80/67 gPa) was characterized initially by activation of Mg-ATPase in the brain (by 17% in the cortex and by 20% in the subcortex) accompanied by a fall of pO_2 by 38% in the cortex and by 28% in the hypothalamus (Table 1; Figs. 1 and 2). Later, after 70 min of hypotension (series III, BP = 73/60 gPa), when pO_2 in the cortex had fallen by 46%, cytosol creatine kinase activity was increased by 54%, the AMP content was increased by almost 1.5 times, and EC was reduced. In the subcortex pO_2 and Mg-ATPase activity were stabilized, whereas mitochondrial creatine kinase activity was increased by 55%, cytosol creatine kinase activity by 49%, and the AMP content by 1.5 times.

In the stage of decompensation (BP = 13/7 gPa), which developed in most (56%) of the untreated animals 60 ± 14 min after blood loss, further deepening of hypoxia took place (pO_2 in the cortex fell by 58% and in the hypothalamus by 48%), cytosol creatine kinase was activated in the cortex by 33% and in the subcortex by 43%, the ATP level fell by 17 and 18%, respective-

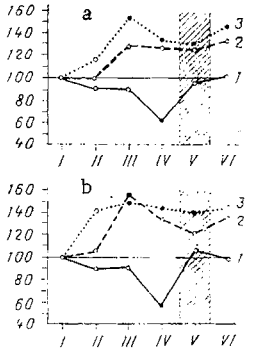

Fig. 2. CP content (1) and activity of mitochondrial (2) and cytosol (3) creatine kinase in cat brain tissue after acute blood loss and HBO. Abscissa, series of experiments; ordinate, CP content and enzyme activity (in % of control). Remainder of legend as in Fig. 1.

ly, EC fell by 13 and 15%, and the CP content by 38 and 43%, whereas the ADP content in the subcortex increased by 29% and AMP in both parts of the brain was more than doubled. This is evidence of depression of energy formation in the "hypoxic" brain, both as energy accumulated in ATP and in the form of its deep reserve, namely CP [6].

During HBO of the anemic animals BP rose to 100/85 gPa, and pO_2 in the cortex increased by 125% at the beginning and by 32% at the end of the session. After decompression, the value of pO_2 in the cerebral cortex fell rapidly during the first 10-15 min and was 42% below its initial values, whereas pO_2 in the hypothalamus remained at its initial level. Meanwhile, during normalization of pO_2 in the hypothalamus, activation of CCO by 34% and of Mg-ATPase by 25% was observed in the diencephalon, while the activity of these enzymes remained at their initial level in the cortex. The ATP content and value of EC in the brain remained the same as in animals of the control series. However, the ADP concentration was increased in the subcortex by 26% and the AMP content was increased by 1.5 times in both parts of the brain. After exposure to HBO the normal CP level was preserved in the sensomotor cortex and diencephalon, whereas cytosol creatine kinase, responsible for utilization of CP in the reaction of ATP synthesis [15], was activated by 30% in the cortex and by 38% in the subcortex, whereas mitochondrial creatine kinase activity showed no significant change.

In healthy animals HBO (BP = 213/200 gPa) increased cytosol creatine kinase activity in the cortex by 46% and increased the AMP content in the cortex and subcortex by nearly 1.5 times.

HBO in anemic animals thus stimulated CCO activity, abolished compensatory activation of mitochondrial creatine kinase, and maintained increased cytosol creatine kinase activity in the diencephalon, stabilized the increased AMP content at the level of the compensatory stage of blood loss, prevented the fall in pO_2, ATP level, value of EC, and CP content in the brain characteristic of the decompensation stage, and ultimately of the development of a terminal state which occurred in most of the untreated animals. It can be concluded from the facts described above that hyperbaric oxygen plays the role of both positive and negative modulator of enzyme systems depending on the metabolic oxygen demand of the cell preceding exposure to HBO.

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CORRELATION BETWEEN CHANGES IN CONTENT AND ACTIVITY OF MICROSOMAL CYTOCHROME P-450 IN RAT LIVER WITH INTENSIFICATION OF LIPID PEROXIDATION DURING STRESS

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It has been suggested that changes in the cytochrome P-450 content and demethylase activity in the microsomal fraction of the liver after hypothermia in rats are connected with intensification of lipid peroxidation (LPO) in the tissues of the animals [2].

In view of data on intensification of LPO in other forms of stress [4, 5, 6], it was decided to study the effect of immobilization, severe physical exertion, and injection of adrenalin on demethylase activity in rat liver microsomes and in their content of cytochromes P-450 and b_5.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-180 g were used. As stressors the animals were subjected to prolonged immobilization [6] and physical exertion (swimming for 3 h at 25°C). Adrenalin was injected intramuscularly in a dose of 800 μg/kg. The microsomal fraction of the liver was isolated by gel-filtration on Sepharose 2B [14]. Protein was determined by the method in [12]. The content of cytochromes P-450 and b_5 was determined spectrophotometrically [13], using extinction coefficients of $E_{450-500} = 91\ mM^{-1} \cdot cm^{-1}$ for cytochrome P-450 and $E_{424-409} = 185\ mM^{-1} \cdot cm^{-1}$ for b_5.

Aminopyrine demethylase activity of the microsomal fraction was estimated from the rate of formaldehyde formation during demethylation of aminopyrine [2].

Lipids were extracted from the liver by Folch's method. The content of hydroperoxides in the lipids was determined by a ferroredoximetric method [1].

EXPERIMENTAL RESULTS

After the action of the stressors and injection of adrenalin, to simulate exposure to stress [7], the cytochrome P-450 level in the rat liver microsomes was lowered. After swimming for 3 h and immobilization of the animals for 4 h the cytochrome P-450 content was reduced by 19 and 24%, respectively (Table 1). The fall in the cytochrome P-450 level after swimming and immobilization was of short duration: By the 25th hour of immobilization the cy-

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