Antioxidant Defense and Lipid Peroxidation in Rat Tissue after Hypobaric Hypoxia

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Posthypoxic period is characterized by increased formation of lipid peroxidation products. Changes in the antioxidant enzyme systems include decreased catalase and glutathione reductase activities and elevated content of reduced glutathione.

Key Words: *hypobaric hypoxia; lipid peroxidation; antioxidant enzymes*

Free-radical lipid peroxidation (LPO) is a universal process accompanying practically all pathological states and determining the degree of cell damage [14]. There is a complex system of cell protection: nonenzymatic antioxidants (tocopherols, glutathione, ascorbate) and antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), and glutathione reductase [3].

Activation of LPO accompanying ischemia of various organs, particularly of the brain results in the accumulation of primary (diene conjugates) and secondary (malonic dialdehyde (MDA), Schiff bases) LPO products in tissues and biological fluids [5]. Activation of LPO also accompanies hypoxia of organs and tissues and, in particular, reoxygenation period [3,14], when the concentration of oxygen and, even worse, its toxic active forms sharply increases against the background of partially destroyed cell structures [13].

Activation of free-radical processes in hypoxia leads to accumulation of LPO products. The state of the antioxidant enzyme system in hypoxia and reoxygenation is less studied. There are only several publications on the effect of hypoxia on the glutathione metabolism enzyme activity [13].

The aim of the present study was to measure the antioxidant enzyme activity and the content of LPO products in rat tissues during reoxygenation.

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MATERIALS AND METHODS

Random-bred male albino rats weighing 170-220 g were used. Hypobaric hypoxia was modeled in a Biocont-B flow chamber (Parus, Moscow) with 40% KOH as a carbon dioxide sorbent. The animals were "elevated" at an altitude of 10,000 m (80 m/sec ascend rate) and 60 min later returned back. They were decapitated 30 min after hypoxia, and internal organs were removed in the cold. Tissue samples were weighted, homogenized, and centrifuged in 10 volumes of physiological saline in an OPN-3 centrifuge at 1400*g* for 10 min with cooling.

The total antioxidant activity was measured by ascorbate-induced LPO. LPO activity was assessed by the content of primary (diene conjugates) and secondary (Schiff bases [6] and MDA [4]) LPO products. The contents of nonprotein SH-groups [11] and activity of SOD [9], catalase [7], GSH-Px [10], and glutathione reductase [2] were also measured.

The data were processed statistically using the Student *t* and nonparametric Wilcoxon tests.

RESULTS

Rats elevated at 10,000 m exhibited the symptoms of moderate hypoxia: decreased motor activity, lateral posture, minor cyanosis, and tachypnea. Thirty minutes after hypoxia the studied parameters did not return to normal: we observed decreased behavioral

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TABLE 1. Content of LPO Products in the Liver, Brain and Blood of Rats Subjected to Hypoxia (*M±m*)

Group	MDA in the brain, nmol/mg tissue (*n*=6)	MDA in the liver, nmol/mg tissue (*n*=6)	MDA in the blood, nmol/ml (*n*=5)	Shiff bases in the brain, rel. units (*n*=5)
Control	0.070±0.004	0.14±0.005	0.075±0.004	106.2±18.2
Hypoxia	0.077±0.006	0.12±0.006	0.097±0.01*	119.2±18.1

Note. Here and in Tables 2-4: *n* indicate the number of animals; *$p<0.05$ compared with the control.

activity in an actometer and open field and impaired conditioned passive avoidance performance [8].

Biochemical tests revealed a tendency toward an increase in brain MDA content (Table 1), whereas in the liver this parameter remained unchanged. Blood concentration of MDA was significantly increased (1.3-fold). The content of Schiff bases in the brain slightly increased after hypoxia, while the concentration of diene conjugates remained unchanged. The rate of ascorbate-induced LPO increased both in the brain and liver (Table 2). In the posthypoxic period, SOD activity slightly increased, while catalase activity decreased (Table 3). The content of nonprotein SH-groups decreased both in the brain and liver, GSH-Px activity remained unchanged, which glutathione reductase activity decreased (Table 4).

Thus, in the posthypoxic period, the content of LPO end-products increased, while undoubtedly reflects general activation of LPO [6], while the concentration of intermediates remained unchanged. No accumulation of MDA in the liver was observed, probably due to washout of MDA from the liver in the early reoxygenation period.

It has been found that LPO activation usually occurs against the background of reduced activity of the antioxidant enzyme system in the brain [14]. In our experiments suppression of the antioxidant enzyme system in the posthypoxic period was confirmed by acceleration of ascorbate-induced LPO. The antioxidant enzyme system includes SOD and catalase decomposing active oxygen forms and metabolic cascade detoxifying lipoperoxides [12]: α-tocopherol, ascorbic acid (reactivated α-tocopherol), and glutathione system consisting of glutathione, GSH-Px, and glutathione reductase (reactivated ascorbic acid) [15]. Thus, catalase activity, which is extremely low in the brain [1], further decreased in the posthypoxic period. Moreover, the content of nonprotein SH groups (predominantly glutathione) decreased due to the inhibition of glutathione reductase [13]. In our experiments no significant inhibition of GSH-Px was found. It should be noted that similarly to other enzymes, GSH-Px activity was measured at optimal pH and excessive substrate concentration. However, posthypoxic period *in vivo* is associated with glutathione deficiency in the brain and possibly acidosis. Under these conditions GSH-Px is rapidly inactivated and the rate of GSH-Px-catalyzed reaction most likely will decrease.

Thus, we cannot exclude the possibility that disturbances in the high integrative activity, in particular, behavioral and mnestic functions in rats subjected to hypoxia are associated with activation of free radical processes in the brain. This is manifested in enhanced generation of LPO end-products

TABLE 2. Rate of Ascorbate-Induced LPO in Brain and Liver Homogenates from Rats Subjected to Hypoxia (*M±m*, *n*=6)

Group	Rate of ascorbate-induced LPO, rel. units	
	brain	liver
Control	0.19±0.013	0.22±0.03
Hypoxia	0.24±0.01*	0.35±0.01*

TABLE 3. Activity of Peroxide- and Superoxide-Decomposing Enzymes in the Brain of Rats Subjected to Hypoxia (*M±m*, *n*=6)

Group	SOD, activity units/mg tissue	Catalase, μmol/min/g tissue
Control	0.40±0.06	233.1±39.0
Hypoxia	0.55±0.05	163.8±33.3

TABLE 4. Content of SH-Groups and Activity of Glutathione-Converting Enzymes in Rats Subjected to Hypoxia (*M±m*, *n*=6)

Group	Nonprotein SH-groups, μmol/g tissue		GSH-Px, μmol/min/g tissue	Glutathione reductase, μmol/min/g tissue
	brain	liver	brain	brain
Control	2.5±0.07	5.4±0.9	38.6±9.3	0.09±0.01
Hypoxia	2.2±0.07*	4.7±0.3	55.0±4.0	0.04±0.01*

(MDA and Schiff bases), acceleration of ascorbate-induced LPO, and suppression of the antioxidant enzyme system, as evidenced by the reduced activity of catalase and glutathione reductase and decreased glutathione content.

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