Effect of Hypoxic Preconditioning on Free Radical Processes in Tissues of Rats with Different Resistance to Hypoxia

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We studied the effect of single hypoxic preconditioning exposure (hypobaric hypoxia, 5000 m, 60 min) on free radical processes, glutathione system, and antioxidant defense enzymes in tissues of rats with different resistance to acute hypoxia. The intensity of free radical processes was shown to increase or decrease on day 1 after hypoxic preconditioning. These changes were tissue-specific and opposite in animals with genetically determined differences in the resistance to hypoxia. Hypoxic preconditioning contributes to the immediate resistance. The effect was more pronounced in low resistant animals, who did not exhibit signs of oxidative stress in tissues during the early posthypoxic period. By contrast, hypoxic preconditioning was followed by activation of free radical processes in tissues of highly resistant animals. These rats were characterized by low ability for the development of immediate resistance. Activation of free radical processes in the early period of adaptation (first hours after hypoxic preconditioning) does not play a role in the induction of immediate adaptive mechanisms for hypoxia.

Key Words: *hypoxic preconditioning; free radical processes; antioxidant defense enzymes; phenotypic resistance to hypoxia*

There is a general agreement that postischemic and posthypoxic states caused by reperfusion and reoxygenation are accompanied by activation of free radical processes (FRP), playing a signal role in the mechanisms of immediate and delayed adaptation. The protective effect of hypoxic preconditioning against hypoxic/ischemic injury is probably associated with activation of FRP [3,4]. Hypoxic preconditioning is the influence of weak (non-damaging) hypoxic or ischemic stimulation, which increases the resistance to subsequent severe hypoxia. This approach is extensively used in medical practice for improving nonspecific resistance of the whole body or organs to adverse factors and extreme conditions and for activation of immediate defense mechanisms of adaptation. Preconditioning prevents the development of functional or metabolic disturbances typical of hypoxia/ischemia. ATP concentration and respiratory control do not differ from normal under these conditions. This procedure prevents impairement of permeability of the outer and inner mitochondrial membranes, changes in membrane potential, release of cytochrome C, or induction of apoptosis [9]. However, the mechanisms underlying the effect of preconditioning are poorly understood. Moreover, some authors believe that the intensity of FRP does not necessarily play a role in the mechanisms of adaptation [5,7,9]. Therefore, this problem requires further investigations.

Here we studied the intensity of FRP and activity of antioxidant defense enzymes in animals with genetically determined differences in the resistance to O_2 deficiency [1].

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MATERIALS AND METHODS

Experiments were performed on male albino rats. One month before the study, the animals were tested for the resistance to acute hypobaric hypoxia in an altitude chamber. The rats were divided into groups of highly resistant (HR, survived in the altitude chamber at the critical height of 11,500 m for at least 10 min) and low resistant (LR, exhibed signs of asphyxia within 1-3 min) animals [2]. These two types of animals (HR and LR) are described by two different functional and metabolic patterns. They are associated with typical differences in activity of CNS and neurohumoral regulation, stress-activating and stress-limiting systems, oxygen-transporting function of the blood, and the state of membranes or receptors [1]. Moreover, these parameters are coupled with energy exchange and functional activity of the respiratory change in animal tissues [1].

Experimental animals were subjected to single session of preconditioning with hypobaric hypoxia in an altitude chamber at 5000 m (10% O_2) for 60 min. The rats were decapitated 1 and 30 min and 1, 2, and 24 h after hypoxic exposure. The neocortex and blood serum were sampled.

For evaluation of the intensity of FRP, the concentrations of hydroperoxides, conjugated dienes of polyunsaturated fatty acids, and thiobarbituric acid-reactive substances (TBA-RS) were measured in homogenates of the neocortex and blood serum [6]. The pool of glutathione (total glutathione, reduced glutathione, and oxidized glutathione) and activity of cytoplasmic enzymes (glutathione peroxidase, glutathione reductase, catalase, and Cu,Zn-containing SOD) were studied in homogenate of the neocortex.

The results were analyzed by the Mann–Whitney and Student *t* tests. The differences were significant at $p<0.05$.

RESULTS

Our previous experiments showed that preconditioning with hypobaric hypoxia increases the resistance of animals to severe hypoxia. This effect was most significant immediately after hypoxic exposure [2]. The resistance of LR and HR rats to hypoxia was shown to increase by 6.5 and 3.5 times, respectively, immediately after 60-min training. The preconditioning-induced immediate resistance to acute hypoxia decreased progressively and was minimum 60 min after preconditioning. However, during this period the resistance of animals exceeded the baseline level by 1.5-3.0 times.

These differences in the sensitivity of animals to hypoxia were not related to baseline characteristics of LPO and activity of antioxidant enzymes. The brain tissue (neocortex) and blood serum in control LR and HR rats did not differ under normoxic conditions (Tables 1 and 2).

Single exposure to hypoxic preconditioning had little effect on the content of TBA-RS and conjugated dienes in the neocortex of LR rats (day 1; Fig. 1). However, the concentration of hydroperoxides decreased significantly 30 min after preconditioning (by 25%, $p<0.05$) and remained low over the next 24 h. TBA-RS content in the neocortex of HR animals tended to increase 2 h after preconditioning (statistically insignificant). The amount of conjugated dienes and hydroperoxides remained practically unchanged under these conditions.

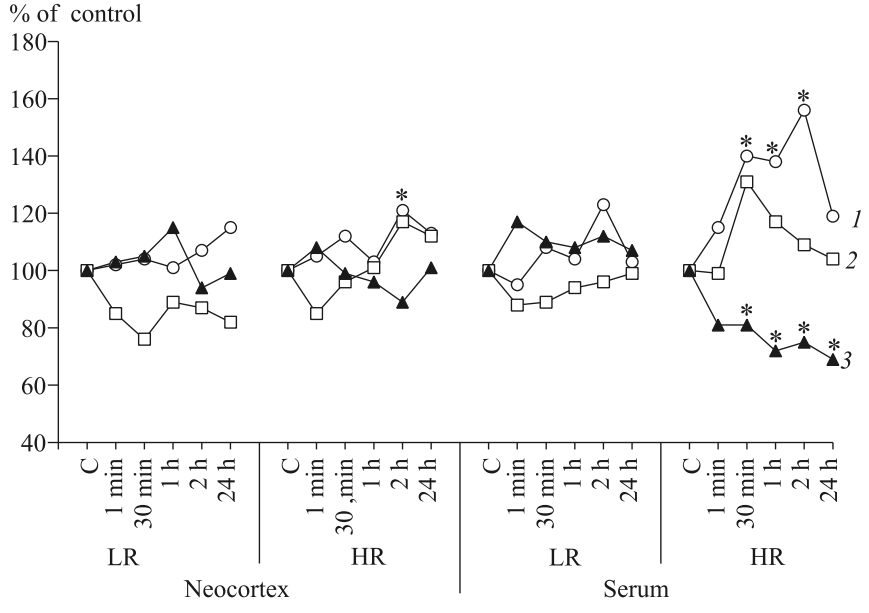

Fig. 1. Effect of hypobaric hypoxic preconditioning on FRP and redox properties of tissues in LR and HR rats. TBA-RS (*1*); hydroperoxides (*2*); conjugated dienes (*3*). Here and in Figs. 2 and 3: *$p<0.05$ compared to the control (C).

TABLE 1. Content of LPO Products in the Brain Cortex and Blood Serum of LR and HR Rats on Day 1 after Hypoxic Preconditioning (*M*±*m*)

Time after preconditioning		LR			HR		
		TBA-RS	HP	CD	TBA-RS	HP	CD
Neocortex	control	434±37	18.2±1.65	20.9±2.8	459±47	18.1±1.70	19.9±2.0
	1 min	441±40	15.4±1.05	21.5±1.9	480±45	15.5±1.46	21.5±1.9
	30 min	452±44	13.9±1.12*	21.9±2.3	513±53	17.4±1.82	19.7±2.1
	1 h	438±38	16.2±1.39	24.2±2.6	475±49	18.3±1.94	19.2±1.8
	2 h	465±42	15.8±1.27	19.7±1.8	555±58	21.2±1.98	17.9±1.9
	24 h	499±46	14.9±1.19	20.8±1.9	520±50	20.2±2.36	20.0±2.6
Blood serum	control	9.7±0.72	17.1±1.81	4.13±0.35	10.1±0.95	13.9±1.24	5.39±0.62
	1 min	9.2±0.90	14.9±1.22	4.82±0.41	11.7±1.06	13.9±1.19	4.37±0.40
	30 min	10.5±1.19	15.3±1.91	4.55±0.50	14.2±1.29*	18.3±1.66*	6.09±0.69
	1 h	10.1±1.37	16.2±1.51	4.45±0.49	13.9±1.15*	16.4±1.5	3.87±0.42
	2 h	11.9±1.09	16.4±1.57	4.61±0.48	15.8±1.37*	15.2±1.39	4.04±0.36
	24 h	9.9±1.02	16.9±1.72	4.40±0.47	12.1±1.09	14.5±1.51	3.70±0.39

Note. TBA-RS, nmol/g(ml); HP: hydroperoxides (cumene hydroperoxide equivalents, g (ml); CD: conjugated dienes, nmol/g (ml). Here and in Table 2: *p<0.05 compared to the control.

Minor changes were found in the amount of LPO products in the serum from LR rats (Table 1, Fig. 1). However, TBA-RS content in the serum from HR rats increased significantly 30 min after hypoxic exposure and remained high over 1 day (by 1.5 times). The observed changes were accompanied by an increase in the concentration of hydroperoxides in the serum of these animals (Table 1, Fig. 1).

Our results indicate that single exposure to hypoxic preconditioning is not necessarily accompanied by activation of free radical LPO in tissues (as observed under conditions of severe or long-term hypoxia) [3,4]. Various tissues are characterized by different reactions to this treatment, which suggests the existence of variations in the equilibrium state of antioxidant defense systems. The early reaction of LPO to mild hypoxia varies significantly in animals with phenotypically different sensitivity to hypoxia. The content of LPO products in the serum increases in HR rats, but remains unchanged in LR animals. The data attest to

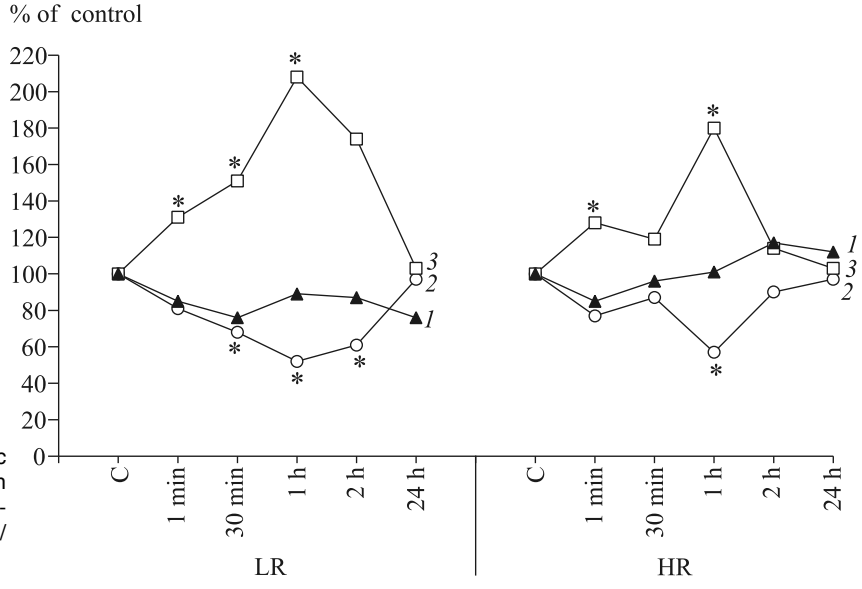

Fig. 2. Effect of preconditioning with hypobaric hypoxia on components of the glutathione system in the neocortex of rats LR and HR to hypoxia. Hydroperoxides (*1*); oxidized glutathione (*2*); reduced/oxidized glutathione (*3*).

TABLE 2. Glutathione Pool and Antioxidant Enzyme Activity in the Brain Cortex of LR and HR Rats on Day 1 after Hypoxic Preconditioning (*M*±*m*)

Time after preconditioning		GSH	GSSG	G	GPx	GR	Cat	SOD
LR	control	2.42±0.19	0.31±0.03	2.73±0.21	31.80±3.44	44.2±4.7	1.97±0.16	16.60±1.78
	1 min	2.57±0.23	0.25±0.02	2.82±0.24	30.90±2.89	44.3±4.6	1.75±0.20	26.90±2.92*
	30 min	2.48±0.29	0.21±0.02*	2.69±0.25	32.60±3.38	48.7±4.9	1.87±0.16	17.90±2.34
	1 h	2.59±0.21	0.16±0.01*	2.75±0.22	39.00±3.64	34.2±3.8	1.41±0.15*	14.10±1.48
	2 h	2.58±0.26	0.19±0.02*	2.77±0.25	34.40±3.26	43.1±4.8	1.23±0.14*	18.10±2.26
	24 h	2.41±0.20	0.30±0.03	2.71±0.26	35.30±3.28	58.2±5.1	1.04±0.12*	30.60±2.73*
HR	control	2.63±0.29	0.30±0.02	2.93±0.23	34.3±3.7	41.7±4.0	1.63±0.14	17.1±1.6
	1 min	2.59±0.27	0.23±0.02	2.82±0.25	33.8±3.4	45.1±4.4	1.58±0.15	24.60±2.2*
	30 min	2.74±0.25	0.26±0.03	3.01±0.27	28.6±3.2	41.6±4.6	1.16±0.12	21.0±2.3*
	1 h	2.69±0.22	0.17±0.02*	2.87±0.23	33.8±3.5	41.4±4.3	1.80±0.16	24.8±2.1*
	2 h	2.71±0.26	0.27±0.03	2.98±0.25	33.3±3.6	43.80±4.72	1.37±0.12	25.8±2.6*
	24 h	2.65±0.27	0.29±0.02	2.94±0.26	30.9±3.3	40.3±3.9	1.39±0.14	25.2±2.1*

Note. GSH, reduced glutathione, μmol/g; GSSG, oxidized glutathione, μmol/g; G, total glutathione, μmol/g; GPx, glutathione peroxidase, nmol NADPH/mg protein/min; GR, glutathione reductase, nmol NADPH/mg protein/min; Cat, catalase, μmol H_2O_2/mg protein/min; SOD, superoxide dismutase, arb. units/mg protein/min.

greater lability of FRP that can be easily induced under conditions of hypoxia in HR animals.

Our previous studies showed that the increase in the resistance to hypoxia is observed immediately after preconditioning [2]. The effect was more pronounced in LR rats, who did not exhibit signs of oxidative stress in the studied tissues during the early posthypoxic period. This probably suggests that FRP do not play a role in the immediate mechanisms of adaptation.

Differences in the tissue response to hypoxic preconditioning in animals of these types were confirmed by studying the main enzymes and antioxidant defense systems. Reduced glutathione is the major intracellular nonenzymatic antioxidant. During LPO activation, this agent plays a role of an electron donor and neutralizes hydroperoxides and lipoperoxides. Otherwise, reduced glutathione scavenges free oxygen radicals. Published data show that the content of reduced glutathione decreases, while the amount of oxidized glutathione

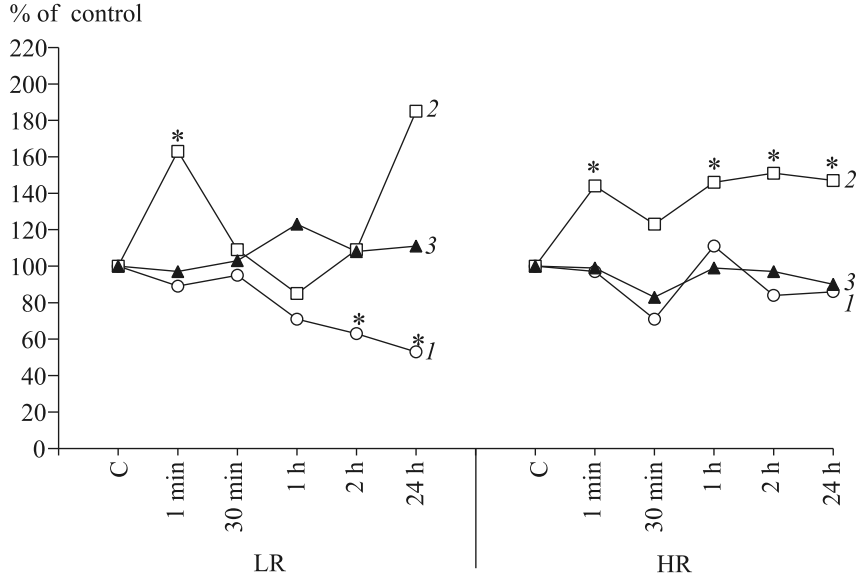

Fig. 3. Effect of hypobaric hypoxic preconditioning on activity of antioxidant defense enzymes in the neocortex of LR and HR rats. Catalase (*1*); SOD (*2*); glutathione peroxidase (*3*).

(marker of oxidative stress) increases during the posthypoxic period [9].

We found that the content of oxidized glutathione in the neocortex from animals of both groups does not increase, but even decreases after preconditioning; increased under these conditions, which reflects more reduced state of the tissue. This effect was particularly pronounced in the cerebral cortex of LR animals over the first 2 h (Fig. 2). No significant changes were found in the total content of glutathione and activities of glutathione peroxidase and glutathione reductase. However, the amount of hydroperoxides decreased under these conditions (Fig. 3, Table 2).

Qualitative changes in the glutathione pool in the neocortex of HR rats were similar, but less pronounced than in LR animals. The content of hydroperoxides decreased over the 1st minute after preconditioning, but increased progressively in the follow-up period (Fig. 3, Table 2).

Differences in the redox properties of brain tissue in animals of two types are associated with functions of the glutathione system and occur in the early period after preconditioning. The antioxidant defense system of brain tissue in LR animals is probably more balanced than in HR specimens. These properties contribute to low intensity of FRP (no changes in TBARS content and decrease in hydroperoxide concentration) over the first hours after hypoxic exposure. The imbalance in this system develops slowly, which results in the appearance of signs for oxidative stress.

As differentiated from LR animals, the antioxidant system is less balanced in the cerebral cortex of HR specimens. This conclusion is derived from minor changes in the reduced/oxidized glutathione ratio and progressive accumulation of hydroperoxides during the early posthypoxic period.

Our suggestion is confirmed by the data on variations in SOD activity in the brain of LR and HR rats. Enzyme activity in LR rats varied significantly during the posthypoxic period. SOD activity was elevated 1 min after hypoxia (by 1.6 times compared to the control), returned to normal by the 30th minute, remained unchanged over 2 h, and increased repeatedly on day 1 (by 2 times). Hence, FRP in the brain of LR rats rapidly return to normal after hypoxic preconditioning. This process is accompanied by changes in enzyme activity. Secondary activation of SOD probably reflects progressive development of oxidative stress.

As differentiated from HR rats, SOD activity in the cerebral cortex of LR animals did not return to normal after preconditioning. Enzyme activity remained high over 1 day, which reflects increased intensity of FRP in the brain tissue during the posthypoxic period (Fig. 3).

The preconditioning-induced changes in LR specimens are probably related to well-balanced function of the glutathione system, which provides efficient antioxidant defense of the brain tissue. This system is suppressed in HR specimens, which contributes to an imbalance in the regulation and maintenance of redox properties of the tissue and low efficiency of brain antioxidant defense.

These data allow us to make the following conclusions.

Single hypoxic preconditioning exposure causes immediate activation or suppression of FRP. These changes are tissue-specific and depend on metabolic processes, redox properties, and prooxidant/antioxidant ratio in tissues.

Hypoxic preconditioning contributes to the development of immediate resistance, which is particularly pronounced in LR animals. These specimens do not exhibit signs of oxidative stress in tissues during the early posthypoxic period. By contrast, in HR animals, characterized by low ability for the resistance development, hypoxic preconditioning is followed by activation of FRP. These rats are. Therefore, activation of FRP in the early period of adaptation does not play a role in induction of immediate adaptive mechanisms.

Hypoxic preconditioning causes opposite changes in oxidative metabolism and antioxidant activity in tissue of animals with genetically determined differences in the sensitivity to hypoxia.

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