# THE PRINCIPLES OF PROTECTIVE EFFECTS FORMATION USING DIFFERENT HYPOXIC PRECONDITIONING MODES

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**The aim of the investigation** was to study molecular mechanisms of adaptive reactions formation using short- and long-term hypoxic preconditioning modes.

**Materials and Methods.** We carried out experiments on white outbred male rats. The animals underwent hypoxic preconditioning by 4- and 28-time hypobaric training within 60 min a day in altitude chamber at 310 mm Hg. Hypoxia tolerance test was performed by simulating severe hypobaric hypoxia in preconditioned animals exposed to atmosphere air rarefied to 143 mm Hg, with 30-minute exposure. We determined the intensity of free radical oxidation processes, catalytic activity of lactate dehydrogenase, neuronal enolase, as well as glucose concentration in brain tissue and blood.

**Results.** The comparison of biochemical measurements in animals in 4- and 28-time trainings and in intact group showed no statistically significant changes in brain tissue and blood. Hypoxia tolerance test in both exercise modes revealed the reduction of glucose concentration, total activity of lactate dehydrogenase, and free radical oxidation processes in brain and blood of animals, though in varying degrees. The neuronal enolase level in blood serum of the exercised animals was within normal range.

**Conclusion.** Metabolic adaptation is a controlled process aimed at homeostasis support under hypoxia. The adaptive mechanism is realized through the remodeling of metabolic state depending on adaptation period duration.

**Key words:** free radical oxidation; hypoxia; hypoxic preconditioning.

Hypoxic and ischemic injuries are the basic to or the contributing factors of the pathogenesis of many diseases. The reduced oxygen delivery to tissues is accompanied by the inhibition of metabolic processes and cell dysfunction [1].

Complicated dynamics of the phenomenon, the involvement of a wide range of functional metabolic systems controlling the phenomenon at different levels of organization determine the diversity of limiting parts and mechanisms underlying hypoxia.

Metabolic changes under oxygen deficiency in biological systems are characterized by glycolysis, lipolysis, and proteolysis activation, metabolic or respiratory acidosis development, mitochondrial swelling, and therefore, uncoupling of oxidative phosphorylation and respiration, ATP deficiency, the weakening of energy-dependent reactions in cells of different structural and functional organization [2].

Energy metabolic imbalance in hypoxia is accompanied by excessive free radical oxidation (FRO) activation [3–5]. Lipid peroxidation reactions lead to the serious disorders of the nervous tissue. The brain contains a large quantity of unsaturated fatty acids, which are the most exposed to peroxide oxidation, and cerebral cells consume oxygen many times as large than other organ and tissue cells [6, 7].

Various hypoxic preconditioning methods are used recently to enhance the nonspecific resistance of the body to hypoxia. Various technique modes are used both in preventive, and therapeutic medicine (hypoxytherapy) [8–10].

The mathematical model approach developed by A.N. Moshkova [11] confirmed the efficiency of a short-term training in hypoxia-adaptation [8, 10, 12–14]. Long-term hypoxic preconditioning within 28 days was shown to contribute to the stable body state. Experimental determination of training regime criteria should be considered as the necessary stage to develop the conditions of adaptation use in medical practice.

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**The aim of the investigation** was to study molecular mechanisms of adaptive reactions formation using short- and long-term hypoxic preconditioning modes.

**Materials and Methods.** We carried out the experiments on white outbred male rats weighting 200–250 g. The animals were kept in standard conditions. When carrying out the investigations, ethical principles were kept inviolate according to European Convention for the protection of vertebrata used for experimental and other scientific purposes (the Convention was passed in Strasburg, 18.03.1986, and adopted in Strasburg, 15.06.2006).

The animals were divided into 6 groups: intact (Int.) — unexposed animals; hypoxia control animals (H12) — severe hypobaric hypoxia simulation in preconditioned animals exposed to atmosphere air rarefied to 143 mm Hg (conventional altitude of 12 000 m) with 30-minute exposure; 4- (Tr4) and 28-time (Tr28) trainings without acute hypoxic exposure followed; 4- (Tr4H) and 28-time (Tr28H) trainings with the following hypoxic "stroke".

The animals underwent hypobaric training in an altitude chamber at 310 mm Hg (conventional altitude of 12 000 m) within 4 or 28 days, with 60-minute daily exposure. Hypoxia tolerance test was performed by simulating severe hypobaric hypoxia in preconditioned animals exposed to atmosphere air rarefied to 143 mm Hg, with 30-minute exposure. We estimated some biochemical parameters in blood plasma and brain of the test animals immediately after hypoxia simulation.

We determined glucose concentration in blood and brain using enzymatic colorimetric technique with deproteinization [15], neuron-specific enolase (NSE) in blood serum — by enzyme immunoassay using DRG Diagnostics test kit (Germany). Lactate dehydrogenase (LDH) activity was assessed spectrophotometrically using pyruvate as a substrate (DiaSystems test kit, Bulgaria).

FRO processes in brain and blood of the rats were estimated by $Fe^{2+}$-induced biochemiluminescence. We studied the following chemoluminescence parameters: S — light sum (reflects radical content $RO_2^{\bullet}$ corresponding to FRO termination; the process is influenced by the substances that show both antioxidant and pro-oxidant effect); Imax — maximum luminescence intensity (reflects the potential ability of a biological object for FRO); K — coefficient (1/S) characterizing antioxidant potential [16].

The data were statistically processed using software package Excel and Statistica 6.0 according to the biomedical statistical recommendations [17].

The findings were represented as M±G, where M — arithmetic mean, G — root-mean-square deviation. We determined the reliability of differences according to Kruskal–Wallis test. Two samplings were considered to belong to different parent entities if $p<0.05$.

**Results and Discussion.** The assessment of oxidative processes by the metabolic activity of glucose and some key enzymes of glycolysis participating in glucose metabolism – NSE, LDH, in brain and blood of the animals showed blood glucose level in control rats exposed to hypoxia to increase of 124% (p=0.006) relative to the intact animals (Fig. 1). The blood glucose content increase resulted from hypoxia has the following underlying general mechanism: "discharge"

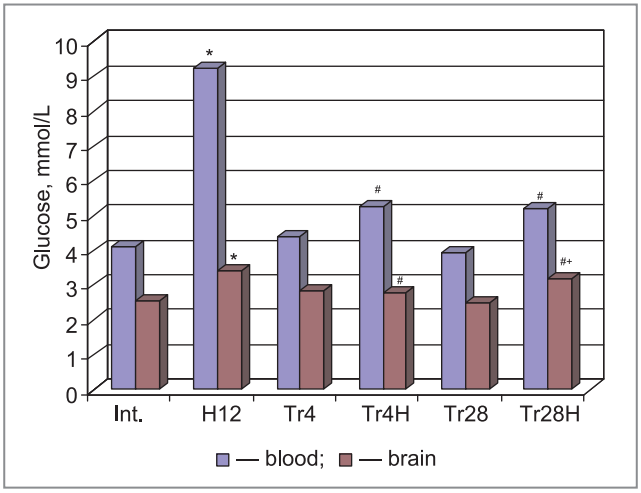


**Fig. 1.** Glucose content (mmol/L) in blood and cerebral tissue of the rats with 4- and 28-day preconditioning. * — statistically significant differences with intact animals, $p\leqslant0.05$; # — with hypoxia control group, $p\leqslant0.05$; + — with group Tr28, $p\leqslant0.05$

of hyperglycemic hormones (catecholamines, glucagon) significantly exceeds the responsiveness of insulin-secretory apparatus [18]. Hypoxic hyperglycemia is accompanied by glucose concentration increase in nervous tissue of 34% (p=0.01) relative to the intact animals. Oxygen deficiency in brain results in rapid changes in metabolic processes, and energy metabolic reactions change first. Under hypobaric hypoxia, total LDH (an amplifier characterizing the energy metabolic status) activity increased of 98% (p=0.009) in cytoplasm of cerebral cells, and of 21% (p=0.009) — in blood compared to the intact animals (Fig. 2). The activation of glucose metabolism results in lactic acid accumulation (according to our findings, blood lactate increases twice) causing acidosis development; and it is related to the LDH activity increase.

Energy metabolic imbalance under hypoxia is accompanied by FRO intensity change in cerebral tissue and blood. The animals under hypoxia were stated to have FRO total activity (S) increase in brain and blood of 28.5% (p=0.017) and 11.8% (p=0.006), respectively (See the Table). Maximum FRO intensity (Imax) also increased of 46.7% (p=0.017) in brain homogenate relative to the norm, and of 16.9% (p=0.018) — in blood plasma. Coefficient K value characterizing the degree of antioxidant protection in the control hypoxia group statistically significantly decreased of 21.9% (p=0.008) in brain homogenate, and of 10.9% (p=0.006) — in blood compared to that of the intact group.

FRO activity increase in nervous tissue is accompanied by the cell membrane structure destabilization. In addition, there is the release of neurospecific enzymes and their isoenzymes from damaged cells into blood [19, 20]. Blood serum of the animals exposed to acute hypoxia was found to have 65% (p=0.004) NSE increase relative to the animals under normal conditions (Fig. 3) that indicates the depth and intensity of structure functional failure of biomembranes in central nervous system.

Thus, acute hypobaric anoxia results in general metabolic changes in the animal' body, and these changes

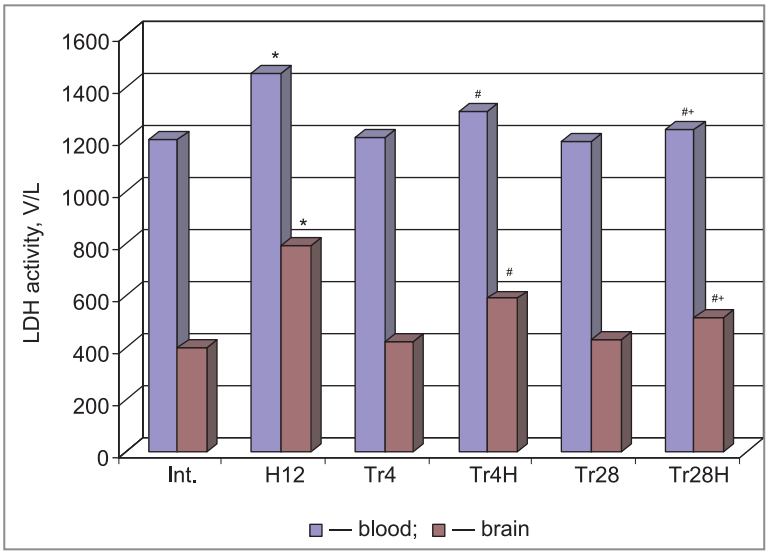


**Fig. 2.** LDH activity in blood and cerebral tissue of the rats with 4- and 28-day preconditioning. * — statistically significant differences with intact animals, p≤0.05; # — with hypoxia control group, p≤0.05; + — with group Tr4H, p≤0.05

**The indices of free-radical oxidation in blood and cerebral tissue of the rats with 4- and 28-day preconditioning**

| Groups | Blood plasma | | | Homogenate of brain | | |
|---|---|---|---|---|---|---|
| | Imax, mV | S, imp./s/mg TL | K = 1/S | Imax, mV | S, imp./s/mg TL | K = 1/S |
| Int. | 580.73±43.71 | 3885.60±262.26 | 2.58±0.18 | 194±4 | 1363.30±37.69 | 7.34±0.20 |
| H12 | 678.78±67.28* | 4342.6±109.9* | 2.30±0.06* | 284.62±18.72* | 1751.52±106.40* | 5.73±0.34* |
| Tr4 | 579.80±24.18 | 3839.30±201.79 | 2.61±0.14 | 190.00±23.04 | 1303.00±14.13 | 7.73±0.75 |
| Tr4H | 536.14±32.60# | 3637.83±79.32# | 2.75±0.06# | 225.20±18.39# | 1548.0±89.9# | 6.48±0.37# |
| Tr28 | 559.11±54.11 | 3949.70±136.06 | 2.53±0.09 | 181.30±26.31 | 1254.42±95.24 | 8.01±0.59 |
| Tr28H | 579.72±21.43#+ | 4054.00±168.48#+ | 2.47±0.11#+ | 208.11±36.70# | 1430.78±211.85# | 7.10±1.01# |

* — statistically significant differences with intact animals, p≤0.05; # — with hypoxia control group, p≤0.05; + — with group Tr4H, p≤0.05.

are characterized by the activation of anaerobic processes and FRO reactions that promote the destruction of membranes and cell death.

Various training regimes have been used recently to increase natural hypoxia resistance of the body [9, 14, 21]. We studied the resistance of rat brain and organism as a whole in various hypoxic preconditioning modes. The comparison of biochemical parameters of the animals with 4- and 28-time trainings relative to the intact group did not reveal statistically significant changes in cerebral tissue and blood. It indicates that the trainings themselves have no negative effect on an animal body; on the contrary, they have a remodeling effect on metabolism reducing its hypoxic component. It is clearly demonstrated when preconditioning time is increased up to 28 days.

The next experimental stage consisted in studying the hypoxia adaptation level of the animals with different preconditioning time.

The glucose level in cerebral tissue of the animals with short-term (4-time) exercises with the following hypoxic stroke was 18.4% (p=0.012) lower and within normal range compared to the animals with hypoxia control. We also found the decrease of total LDH activity in brain of 26% (p=0.006). It can be due to the activation of glucose disposal pathways that indicates the readiness and formation of hypoxia-resistance of the body.

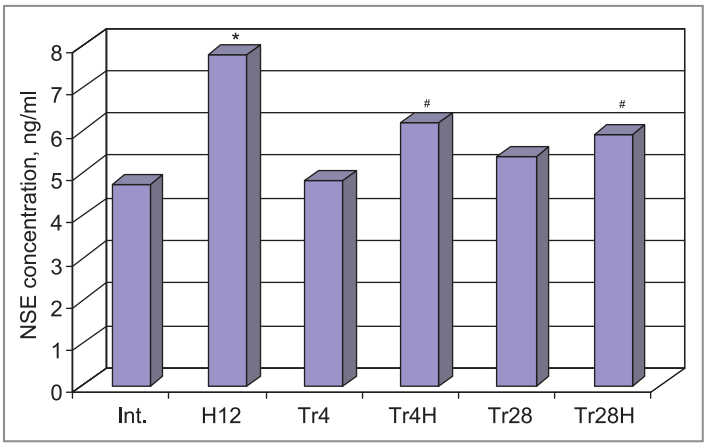


**Fig. 3.** NSE concentration in blood serum of the rats with 4 and 28-day preconditioning. * — statistically significant differences with intact animals, p≤0.05; # — with hypoxia control group, p≤0.05; + — with group Tr28, p≤0.05

The intensity of free-radical processes in nervous tissue in this group of animals decreased of 11.6% (p=0.019) relative to those with hypoxia control, and antioxidant potential (K) increased of 13% (p=0.015), i.e. the proportion of pro- and antioxidant factors in cerebral tissue of the trained animals with the following hypoxic stroke was recovered up to the normal values.

The decreased activity of free-radical processes results in a reduced negative effect of free radicals on the membranes of nervous tissue cells that leads to the reduction of their fluidity and permeability. This is shown by NSE level in blood serum of the animals with short-term exercises with the following hypoxic exposure, and it being 20.4% (p=0.006) lower than that in the group with hypoxia control.

The comparison of blood values in the trained animals after hypoxic stroke with those in the hypoxia control group showed the glucose reduction of 43% (p=0.006) that corresponded to normal values. Total LDH activity in blood plasma in this group also decreased of 10% (p=0.006) compared to the control. Total activity of FRO processes in blood was 16.2% (p=0.004) lower. Antioxidant potential (K) in blood increased of 19.6% (p=0.004).

Thus, 4-time interval hypoxic preconditioning results in energy metabolic transformation aimed at the efficiency increase of energy production and utilization in cells, due to which the tolerance of the rats to the following acute oxygen deficiency increases.

Acute hypoxia tolerance test in 28-day adaptation animals revealed that the glucose level in cerebral tissue was 6.4% (p=0.03) lower than that in the hypoxia control group, and exceeded the norm of 14.2% (p=0.021). There was the decrease of total LDH activity in cytoplasm of brain cells of 35% (p=0.002) relative to the untrained animals, and was significantly lower than in the rats with 4-day trainings.

FRO intensity in the brain of this group of trained animals did not statistically significantly differ from the animals with short-term exercises, and was 18.3% (p=0.017) lower relative to S index of the non-adaptive animals after hypoxia. Antioxidant activity increased of 24% (p=0.016) that indicates the maintenance of cerebral tissue resistance to oxygen deficiency.

HSE level in blood serum in the animals with long-term exercises was 24% (p=0.004) lower than that in the hypoxia control group; it was due to the stabilization of nervous tissue cell membrane structure resulted from the organism adaptation improvement of the animals of this group.

The blood glucose concentration in the 28-day adaptation animals after hypoxic exposure was reduced of 43% (p=0.006) relative to the hypoxia-exposed animals, and corresponded to the normal values. Blood plasma LDH activity in 28-day exercise decreased of 14.6% (p=0.006) relative to the control group, and — of 5.3% (p=0.01) relative to the animals with 4-day exercises.

Free-radical processes in blood of the group relative to that with hypoxia control had the tendency for reduction, however, compared to the animals with short-term training, FRO activity level was statistically significantly higher of 11.4% (p=0.004), and K coefficient decreased of 10.2% (p=0.004).

The findings show that the long-term interval hypoxic preconditioning stabilizes metabolic processes at a new level, and it may be due to the initialization of long-term adaptive mechanisms, pituitary-adrenal system activation, the synthesis of protective proteins, and the change of kinetic properties of oxidative metabolic enzymes that contribute to the efficiency increase of glycolysis and glucose transport through hematoencephalic barrier [8, 22]. Due to the change of these processes, there develops the complex of stable adaptive traits responsible for continuing increase of the body resistance to hypoxia.

**Conclusion.** Experimental findings indicate that interval hypoxic preconditioning has a protective effect on animals. The training time increase promotes the antioxidant protection degree and has a stabilizing effect on cerebral cell membranes. It suggests that metabolic adaptation is a controlled process aimed at homeostasis support under hypoxia. The adaptive mechanism is realized through the remodeling of metabolic state depending on adaptation period duration.

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