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COMPLEX SYSTEMS BIOPHYSICS

The Effects of Severe Hypobaric Hypoxia and Inhibition of Hypoxia-Inducible Factor-1 (HIF-1) on Biomarkers of Cardiac and Skeletal Muscle Injury in Rats

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Abstract—The goal of the present study was to investigate the molecular mechanisms that underlie heart and skeletal muscle damage in male Wistar rats weighing 200–250 g in response to a 3-h exposure to 180 mm Hg (5% O_2) in the model of severe hypobaric hypoxia. It has been demonstrated that the level of the cardiac biomarker troponin I in the blood plasma of rats exposed to severe hypobaric hypoxia for 3 h increased significantly compared to the control group, indicating myocardial injury. At the same time, the administration of the HIF-1α transcription factor inhibitor did not affect the plasma level of troponin I. In contrast, the release of the non-specific biomarker myoglobin into the bloodstream did not increase in response to hypoxia compared to the control animals. In addition, 24 h after the exposure to severe hypobaric hypoxia the serum myoglobin level was significantly lower in animals administered with the HIF-1α inhibitor topotecan than in rats that did not receive topotecan. Therefore, it may be assumed that the inhibition of the HIF-1α transcription factor 10 min before exposure to severe hypobaric hypoxia reduces skeletal muscle damage. The mechanisms that affect the adaptation of heart and skeletal muscles to hypoxia are discussed.

Keywords: hypoxia, myoglobin, troponin I, topotecan, hypoxia-inducible factor 1-alpha (HIF-1α), myocardium

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INTRODUCTION

It is known that in aerobic organisms a reduced oxygen supply leads to functional impairments and that under the complete absence of oxygen cells undergo cell death. An adequate oxygen supply is of primary importance for specialized systems such as the nervous system, as well as the cardiovascular, and respiratory systems. Disturbances of this type are observed during high-altitude climbs, i.e., under the conditions of hypobaric hypoxia. The model of hypobaric hypoxia is widely used in studies of the molecular mechanism of neuronal damage in the central nervous system [1]. The cells of multicellular organisms evolved the capacity to "sense" reductions in the availability of oxygen and to undergo adaptive changes in gene expression that either enhance oxygen consumption (that facilitates increased oxygen supply) or promote adaptation to hypoxic conditions. The Hypoxia Inducible Factor (HIF) transcription factor family plays a key role in this process. One of the proteins of this family, HIF-1, activates genes that regulate glucose transport and glycolysis, thus providing cell adaptation to hypoxia, and also enhances the expression of the vascular endothelial factor and its receptors in response to a reduced oxygen supply (hypoxia) [2]. HIF-1 may also exhibit an adverse effect by contributing to the development of post-hypoxic pathologies [3, 4].

HIF-1 is a heterodimer that consists of an unstable alpha-subunit (HIF-1α) and a stable beta-subunit (HIF-1β). The dimer associates with the DNA in two specific regions called hypoxia-responsive elements. When the oxygen supply is adequate, HIF-1α is hydroxylated on either the first or second highly conserved lysine residues by members of the prolyl hydroxylase domain-containing protein family [5]. This results in the formation of a binding site for a protein component of the ubiquitin-ligase complex and leads to polyubiquitination and further proteasomal degradation of HIF-1α if there is sufficient oxygen (normoxic conditions). In moderate hypoxia, the rate of HIF-1α hydroxylation decreases; therefore, it undergoes proteasomal degradation at a slower rate, accumulates, dimerizes with HIF-1β, and then is imported into the nucleus, where it activates the tran-

Abbreviations: HIF, hypoxia-inducible factor; NO, nitrogen monoxide.

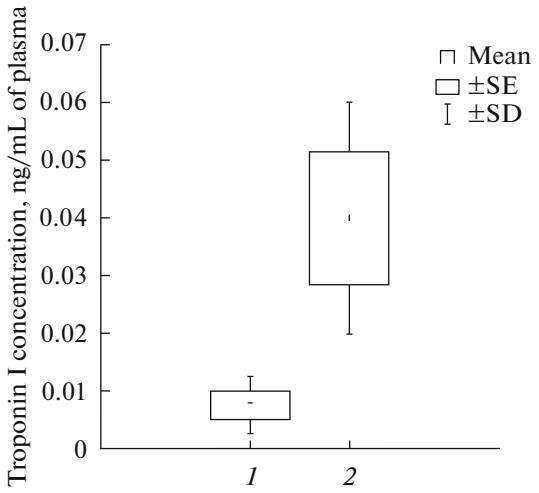

Fig. 1. The alterations of the troponin I level in blood plasma of rats that were not exposed (*1*) and exposed (*2*) to 3-h severe hypoxia, 24 h after the hypoxic session. The ordinate shows the concentration of troponin I in ng per 1 mL of blood plasma.

scription of 100 to 200 genes, including those that participate in erythropoiesis, angiogenesis, autophagia, and energy metabolism. There is another protein family, called factors inhibiting HIF-1, that acts similarly to the prolyl hydroxylase domain protein family but at lower oxygen concentrations [2, 6].

In recent years, it became apparent that the regulatory mechanisms of HIF-1 activity are affected by various factors, including reactive oxygen species and nitrogen oxide [2]. Therefore, in the present study, an attempt was made to investigate the effects of HIF-1 transcription inhibition on skeletal and cardiac muscle damage caused by severe hypobaric hypoxia.

MATERIALS AND METHODS

The model of severe hypobaric hypoxia, that is, exposure of male Wistar rats weighing 200–250 g to 180 mm Hg (5% O_2) for 3 h [7], was used in this study. Topoisomerase I inhibition with topotecan [8, 9] was used to assess the role of HIF-1. Topotecan was administered with a "dimethyl sulfoxide–0.09% NaCl" mixture intraperitoneally (5 mg/kg body weight) 10 min prior to each hypoxic session. The control animals were administered with a "dimethyl sulfoxide–0.09% NaCl" mixture only. To measure the concentration of a specific biomarker of cardiac muscle injury, that is, cardiac troponin I, the level of a less specific cardiac muscle injury biomarker, myoglobin, and the level of total protein in blood plasma blood samples were taken from rats after a session of severe hypoxia. The blood plasma was frozen at –80°C until it was used to determine the levels of biomarkers (myoglobin, troponin I) using a DXI chemiluminescent immunoassay analyzer (Beckman Coulter, United States). The level of troponin I was analyzed using the Access TnI A 78803 toolkit, and the level of myoglobin was analyzed using the Access Immunoassay Systems Myolobin 973243 toolkit manufactured by Beckman Coulter. The total protein was determined by the Biuret method using a DXC Unicell biochemical analyzer (Beckman Coulter, United States). The myoglobin concentration was calculated in proportion to the total protein concentration. The data on biomarker levels were obtained in two independent experimental sessions, each of which included up to six animals. The data processing was performed using the analysis of the variance (the test for significance of difference between means in small samples [10]). The figures were prepared using StatSoft Statistica 6.0 software.

RESULTS AND DISCUSSION

The results demonstrate a significant increase in the level of cardiac troponin I in the blood plasma of rats exposed to 3-h hypoxia (Fig. 1), which indicates myocardial injury. Moreover, the inhibition of HIF-1α transcription before the hypoxic session did not affect the result (the data are not presented).

It appears that HIF-1 does not affect myocardial injury and this damage is most probably associated with the effect of reactive oxygen species produced in the myocardium in response to severe hypobaric hypoxia and reoxygenation that follows hypoxia. As noted in the Introduction, reactive oxygen species may influence the mechanisms of HIF-1 activity regulation in hypoxia, that is, hydroxylation via proteins of the prolyl hydroxylase family and the family of HIF-1 inhibiting factors [11, 12].

The second component that could both directly influence the cardiomyocyte components and affect the regulatory mechanisms of HIF-1 activity is nitrogen monoxide (NO).

In normoxic conditions, NO causes a stabilization of hypoxia-inducible factor, probably via the direct inhibition of the hydroxylation reaction catalyzed by the domain of prolyl hydroxylase domain proteins.

In hypoxia, NO attenuates HIF-1α induction (probably by reducing mitochondrial oxygen consumption), thus increasing the availability of intracellular oxygen [13].

Nitrogen oxide is a key component in the regulation of hemodynamic parameters, such as peripheral resistance, arterial blood pressure, myocardial contractility, heart rate, and, therefore, tissue perfusion [14]. The primary source of NO in normoxia is various isoforms of nitric oxide synthase. However, in hypoxic conditions, NO-synthase becomes ineffective because the activity of these enzyme family members depends on oxygen. In hypoxic conditions, the role of nitrite, which is the second major source of NO, increases [15, 16]. It has been found that hypoxia facilitates the reduction of nitrite to NO catalyzed by nitrite reduc-

tases that function as a complement to oxygen-dependent NO-synthases. The produced nitrogen monoxide, in turn, acts as a vasodilator on the muscle vessels and increases the oxygen supply of the tissues [17].

Nitrite reductases primarily include myoglobin, whose level was elevated in the heart, where it exhibits the maximum activity as nitrite reductase [18] and hemoglobin and xanthine oxidoreductase present in erythrocytes. Cytoglobin and the mitochondrial electron transport chain also exhibit nitrite reductase activity with the last acting as nitrite reductase both in hypoxia and normoxia. Further experiments with cardiomyocytes demonstrated that only NO production in mitochondria regulates the level of cyclic guanosine monophosphate involved in cardiac contractility [13].

In the light of the data on an increased cardiac troponin I release into the bloodstream in response to severe hypobaric hypoxia, it would be interesting to know whether any of the cardiac nitrite reductases is damaged in severe hypoxia. It is known that substances that alter the enzyme activity of xanthine oxidoreductase, mitochondrial respiration, and inhibit hemes and thiols, to some extent decrease the conversion of nitrite to nitrogen monoxide in hypoxic conditions [14].

No significant changes in the release of myoglobin, which is a nonspecific biomarker for muscle damage, into the bloodstream were observed after 3 h of severe hypoxia (the data are not presented). This may be explained by a lower increase in the level of reactive oxygen species in skeletal muscle compared to cardiomyocytes since mammalian skeletal muscle tissue predominantly consists of white fibers with a low content of myoglobin and cytochromes, which are characterized by nonoxidative form of metabolism. Therefore, during severe stress conditions, such as hypoxia, the concentration of reactive oxygen species in skeletal muscle fibers increases considerably less than in cardiac muscle and they are less sensitive to hypoxia [19, 20].

The results of the experiment with HIF-1α inhibitor topotecan demonstrated that 24 h after the session of severe hypoxia, myoglobin concentration (in ng per 1 mL of rat blood plasma) was 2.2 times lower than in the experiments without the inhibitor. The results first were not very significant due to the high variation of the values in a sample, but after the standardization and calculation of the concentration of myoglobin in proportion to the total protein concentration, the mean difference became significant (Fig. 2). For troponin I, the mean difference was significant both for the concentration expressed in ng/1 mL and for the concentration in μg calculated in proportion to 1 g of total plasma protein. Therefore, it may be assumed that the inhibition of transcriptional factor HIF-1 during severe hypoxia reduces skeletal muscle damage. It should be mentioned that the author of [20] suggested that cellular hypoxia and HIF-1α do not play a major role in the adaptation of skeletal muscle to hypoxia [17]. This is the reason that the observed decrease in muscle damage is probably a side effect of an inhibitor used in the experiment. With regard to the data on the protective action of HIF-1 inhibition during severe hypoxia, it has been recently found [4] that such inhibition leads to a delayed decline in glucose-6-phosphate dehydrogenase activity in the hippocampus; at the same time, it promotes the elevation of the reduced nicotinamide adenine dinucleotide phosphate level, normalization of the intracellular levels of total glutathione, and the reduction of oxidative stress and cell death. Similar processes probably occur in muscle tissue. In further studies, it would be worth examining the effect of topotecan administration on the function of pulmonary tissue which is more vulnerable to hypoxia-induced damage.

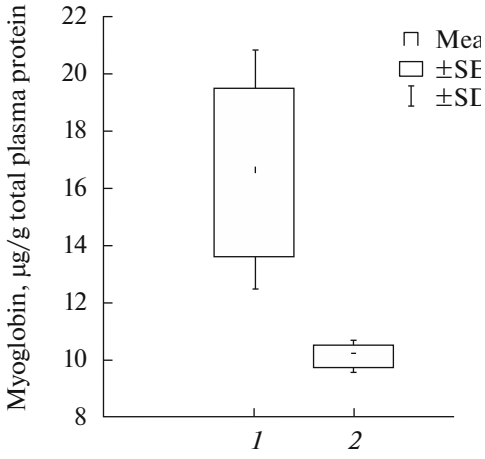

Fig. 2. The alteration of myoglobin concentration in blood plasma of rats exposed to severe hypoxia without (*1*) and with (*2*) topotecan, 24 h after the hypoxic session. The ordinate shows the concentration of myoglobin in μg in proportion to 1 g of total plasma protein.

Therefore, it may be assumed that severe hypoxia adversely affects the heart, while the inhibition of the HIF-1 transcription factor with HIF-1α inhibitor topotecan 10 min before the exposure to severe hypoxia reduces skeletal muscle damage. These results should be considered preliminary and need to be examined using other methods.

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COMPLIANCE WITH ETHICAL STANDARDS

All the international, national, and institutional guides for the care and use of laboratory animals were followed.

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