

Moderate intermittent hypoxia/hyperoxia: implication for correction of mitochondrial dysfunction

Research Article

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Abstract: The purpose of this study was to appreciate the acute hypoxia-induced mitochondrial oxidative damage development and the role of adaptation to hypoxia/hyperoxia (H/H) in correction of mitochondrial dysfunction. It was demonstrated that long-term sessions of moderate H/H [5 cycles of 5 min hypoxia (10% O₂ in N₂) alternated with 5 min hyperoxia (30% O₂ in N₂) daily for two weeks] attenuated basal and Fe²⁺/ascorbate-induced lipid peroxidation (LPO) as well as production of carbonyl proteins and H₂O₂ in liver mitochondria of rats exposed to acute severe hypoxia (7% O₂ in N₂, 60 min) in comparison with untreated animals. It was shown that H/H increases the activity of glutathione peroxidase (GPx), reduces hyperactivation of Mn-SOD, and decreases Cu,Zn-SOD activity as compared with untreated rats. It has been suggested that the induction of Mn-SOD protein expression and the coordinated action of Mn-SOD and GPx could be the mechanisms underlying protective effects of H/H, which promote the correction of the acute hypoxia-induced mitochondrial dysfunction. The increase in Mn-SOD protein synthesis without changes in Mn-SOD mRNA level under H/H pretreatment indicates that the Mn-SOD activity is most likely dependent on its posttranslational modification or on the redox state of liver mitochondria.

Keywords: *Intermittent hypoxia/hyperoxia • Acute hypoxia • Mitochondrial dysfunction • Mn-SOD expression*

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1. Introduction

Oxidative stress is a common and fundamental cause of a wide range of pathophysiologic conditions [1]. Mitochondria can represent a significant source of reactive oxygen species (ROS), which in addition to cytosolic NAD(P)H oxidase and xanthine oxidase contribute to the vicious cycle of ROS production [2]. Recent findings pointed out that severe hypoxia could cause cellular oxidative stress with consequent damage to lipids, proteins, and DNA [3,4]. Oxidative damage to mitochondrial membranes, enzymes, electron transport chain components, ATP production, permeability transition pore opening, and imbalance in the antioxidant systems lead to mitochondrial dysfunction [5]. Dysfunctional mitochondria will produce more ROS and cause additional damage [4]. The monitoring of the changes in mitochondrial pro- and anti-oxidant balance deserves special attention, due to the intensity

of mitochondrial free radical oxidation and antioxidant capacity as well as the influence of these processes on the functional and structural integrity of mitochondria and on the redox regulation of many cellular functions.

Although O₂ is generated from several sites in the mitochondria, the main source of mitochondrial O₂ appears to be within the inner mitochondrial membrane, at complexes I and III of the electron transport chain in particular [6]. Superoxide is transformed to hydrogen peroxide by the detoxification enzymes (Mn-SOD in matrix or Cu,Zn-SOD in intermembrane space [7]) and then to water by glutathione peroxidase (GPx) or peroxiredoxin III and V. However, when these enzymes cannot convert ROS fast enough or act not in concordance, oxidative damage occurs and accumulates in the mitochondria [8].

Mitochondrial ROS generation and antioxidant capacity are potential targets for pharmacological and molecular approaches to correct oxidative stress-induced injuries, including mitochondrial dysfunction.

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In this study, we suggested an alternative approach instead of the standard antioxidant therapy. This approach was to induce the own protective antioxidant system of an organism by applying a new regime of adaptive training, which combines periods of hypoxia and hyperoxia (H/H).

Clinical and experimental studies have confirmed beneficial effects of intermittent hypoxic training (IHT) on hypoxic ventilatory response, an increase in the red blood cell mass, erythropoietin level, and aerobic capacity as well as enhance mRNA myoglobin, hypoxia-inducible factor-1, and vascular endothelial growth factor expression in human muscle after acute exercise [9,10]. Investigations performed in our and other laboratories have shown that adaptation to IHT as well as hyperbaric oxygenation (HO) could reduce the oxidative stress-induced damage caused by extremal influences such as ischemia, exhaustive physical exercise, more severe and sustained hypoxia, and emotional stress [11-15].

The basis of these effects is moderate periodic generation of free radical signal during changes in the oxygen level which induced the activation of various metabolic processes and may be an important trigger for specific adaptation [16]. It is known that the periodic activation of ROS during IHT and HO is involved in a cascade of intracellular redox signaling with subsequent activation of redox-sensitive transcription factors and genes controlling the synthesis of protective components (including antioxidants), which improves the organism's resistance to oxidative stress [17]. H/H is characterized by upregulation of adaptive ROS signals compared to classical IHT and HO and therefore represents an interesting approach for the formation of protective reactions.

Many studies have explained the important role of Mn-SOD in the prevention of oxidative stress [18-20]. However, little is known about the gene and protein expression of Mn-SOD during H/H and its participation in correction of mitochondrial dysfunction.

The present study was thus designed to evaluate the effect of prolonged intermittent moderate H/H on prooxidant/antioxidant balance in liver mitochondria of rats exposed to acute severe hypoxia. Investigation of the indices of lipid peroxidation (LPO), protein carbonyl content, H_2O_2 production, as well as the activity of key antioxidant enzymes such as Cu,Zn-, Mn-SOD, and GPx permit us to appreciate the acute hypoxia-induced mitochondrial oxidative damage development and the role of adaptation to H/H in correction of mitochondrial dysfunction. In the present study mRNA, protein expression, and the specific activity of Mn-SOD at different oxygen levels as important biomarkers of oxidative stress were analyzed.

2. Experimental Procedures

2.1 Materials

All chemicals were purchased from Sigma, Fluka and Merck and were of the highest purity.

2.2 Animals and study design

Male Wistar rats weighing 220-260 g were used. The experimental animals were housed in Plexiglas cages (4 rats per cage) and kept in an air-filtered and temperature-controlled (20-22°C) room. Rats received a standard pellet diet and water ad libitum and were kept under an artificial light-dark cycle of 12 h. The present study was approved by the Animal Ethics Committee at the Bogomoletz Institute of Physiology, Kiev, Ukraine (Protocol No 5/17). Rats were randomly divided into four groups (eight animals in each). Animals of group 1 were kept under normoxic conditions and served as a control. In group 2, rats were exposed to a single action of acute hypoxia by breathing with the hypoxic gaseous mixture (7% O_2 in N_2) for 60 min. Group 3 included animals subjected to sessions of intermittent hypoxia/hyperoxia. We applied repeated short-term 5-min inhalation of gaseous mixture containing 10% O_2 in N_2 with 5-min intervals of hyperoxia (breathing with the hyperoxic gaseous mixture containing 30% O_2 in N_2) under normobaric condition in a special chamber where the temperature and humidity were maintained at 21-26°C and 55-60%, respectively. Rats had five similar sessions daily for 14 days. The gaseous mixtures with low and high content of O_2 were obtained with the help of a device ("Metaks" Co) operating on the membrane gas partition principle with on-line computer control of O_2 levels in isolated animal cages. Rats of group 4 were exposed to acute hypoxia on the first day after cessation of the intermittent hypoxia/hyperoxia training course. Animals were decapitated immediately after the experiment. At the time of sacrifice, the rats were lightly anesthetized with ether.

2.3 Mitochondria isolation

Rat liver mitochondria were isolated by differential centrifugation as described by Jonson and Lardy [21], with some modifications. Liver was collected in isolation medium A (250 mM sucrose, 10 mM Tris/HCl (pH 7.6) and 1 mM EGTA) and homogenized. After centrifugation of the homogenate at 1000xg for 5 min, the supernatant was strained on gauze and recentrifuged at 12000xg for 15 min. The resulting pellet was resuspended in ice-cold isolation medium B (250 mM sucrose, 10 mM Tris/HCl (pH 7.6) and 0.1 mM EGTA) and a new series of centrifugation was performed. The final washing and resuspension of mitochondria was in medium B

without EGTA. Mitochondrial protein concentration was estimated by the Lowry method, using bovine serum albumin as a standard.

2.4 Biochemical assays

The mitochondrial preparations were analyzed after solubilization in 0.5% deoxycholate for 60 min at 0–4°C.

Lipid peroxidation in isolated mitochondria was measured from the formation of thiobarbituric acid - reactive substances (TBARS) using the method of Buege and Aust [22]. Sensitivity to *in vitro* LPO was estimated by incubating identical mitochondria samples with 10 µM FeSO₄ and 0.1 mM ascorbic acid at 37°C for 30 min. The reaction was stopped by addition of 20% TCA.

Protein carbonyls were detected by their reaction with 2,4-dinitrophenylhydrazine (DNPH) leading to formation of protein hydrazones [23]. Absorbance of the samples was measured at 370 nm. Carbonyl contents were calculated using the molar extinction coefficient of DNPH, $\epsilon=22000 \text{ M}^{-1} \text{ cm}^{-1}$.

Intramitochondrial production of H₂O₂ was tested in the lactoperoxidase/H₂O₂/iodide system according to Huwiler and Kohler [24].

Manganese- and Cu,Zn- superoxide dismutase (Mn-SOD and Cu,Zn-SOD) activity was estimated by the method of Misra and Fridovich [25], which is based on the inhibition of autooxidation of adrenaline to adrenochrome by SOD contained in the examined samples. The mitochondrial samples were preincubated at 0°C for 60 min with 6 mM KCN, which produces total inhibition of Cu, Zn-SOD activity. The activity of Cu, Zn-SOD was calculated as the difference between the total SOD activity (without KCN) and the Mn-SOD activity (with KCN). The results were expressed as specific activity of the enzyme in units per mg protein. One unit of SOD activity being defined as the amount of protein causing 50% inhibition of the conversion rate of adrenaline to adrenochrome under specified conditions.

Activity of selenium-dependent glutathione peroxidase (GPx) was measured following NADPH oxidation at 340 nM in the presence of the glutathione reductase excess, reduced glutathione and hydroxide peroxide by the methods of Flohe and Gunzler [26].

2.5 Protein level determination of MnSOD by Western Blot

Isolated mitochondrial protein extracts (100 µg) were separated on SDS-polyacrylamide gel (12%) according to Laemmli [27] and transferred to polyvinylidene fluoride membranes by a semi-dry electrophoretic transfer. The membranes were then blocked with 5% nonfat dry milk in Tris Buffered Saline Tween-20 (TBST) buffer (50 mM Tris/HCl, 150 mM/L NaCl, and 0.1% Tween, pH

7.4) for 1 h at 37°C. Mn-SOD proteins were detected using primary monoclonal antibody for Mn-SOD (Sigma-Aldrich Co) at a dilution 1:1000 for 2 h at 37°C, followed by incubation with horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich Co) (1:2000) for 1 h at 37°C. Each antigen-antibody complex was visualized by amino-ethylcarbazol reaction. The Mn-SOD band intensities were quantified by densitometry with a computerized image processing system (GelPro Analyzer). Results were expressed as percentages of control values.

2.6 RNA extraction and RT-PCR for MnSOD analysis

Total RNA was isolated using the commercial kit "Trizol RNA Prep100" (Isogen, Russia) according to the manufacturer's instruction. Reverse transcription (RT) was performed using RevertAid™ H Minus First Strand cDNA synthesis kit (Fermentas, Lithuania), 1.2–1.5 µg of total RNA and Random hexamer primers. Obtained one-strand cDNA was used for real-time PCR. PCR primers for Mn-SOD and glyceraldehyde-3-phosphatedhydrogenase (GAPDH) were purchased from "Fermentas" (Lithuania) and contained the following sequences: Mn-SOD sense primer: 5' -CTGAGGAGAGCAGCGGTCGT-3', MnSOD antisense primer: 5' -CTTGGCCAGCGCCTCGTGGT-3'; GAPDH sense primer: 5' -GGGTGTGAACCACGAGAAAATATGA-3', GAPDH antisense primer: 5' -AGCACCAGTGGATGCAGGGGATGAT-3'. Mixture for amplification contained 5 µL of 5x PCR buffer, 1.5 mM magnesium sulphate, 0.2 mM of each dNTP, 3 µL cDNA, 1 U Tag-polymerase (AmpliSens, Russia), 30 pM of each of the primers and deionized water to 25 µL of total volume. The mixture was subjected to 40 cycles for Mn-SOD and 30 cycles for GAPDH of sequential steps in an automated thermal cycler "Applied Biosystems 2700" (Perkin Elmer, USA): denaturation (1 min at 94°C for Mn-SOD and GAPDH), annealing (40 s at 61°C for Mn-SOD and 50 s at 65.5°C for GAPDH), then elongation (1 min at 72°C for Mn-SOD and GAPDH). This program was complete with a final extension for 7 min at 72°C. After amplification, the products were separated by electrophoresis on 1.6% agarose gels, ethidium bromide-stained bands were visualized by UV transillumination (BioCom, Russia), and the fluorescence intensity was quantified using a Gel Doc 2000 system (BioRad). There were no significant differences in intensity of GAPDH levels between experimental groups.

2.7 Statistical analysis

Data are expressed as means ± SEM for each group. The differences among experimental groups were

detected by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test.

3. Results

3.1 Lipid peroxidation, protein carbonyl content, and hydrogen peroxide production

Figure 1 shows that acute hypoxia significantly affected the intensity of oxidative processes in liver mitochondria. We registered the increases in basal and stimulated LPO by 64% and 45%, respectively ($P < 0.05$). Moreover, protein carbonyl content and H_2O_2 production were significantly elevated by 46% and 30%, respectively, as compared to control ($P < 0.05$) (Figure 2). Sessions of H/H did not cause any significant changes in the oxidative proteins level. However, we observed in these

samples the increases of TBARS content and H_2O_2 production. We demonstrated an increase in basal LPO level in the mitochondrial fraction as well as in LPO following an addition of exogenous inducers by 19–21% in comparison with control group 1 ($P < 0.05$). Acute hypoxia after H/H treatment (group 4) induced a decrease in TBARS contents of basal and stimulated LPO by 33% and 15%, respectively, as compared with group 2 ($P < 0.05$). In addition, H_2O_2 production and carbonyl protein concentration decreased by 38% and 10%, respectively, in comparison with untreated rats ($P < 0.05$).

3.2 Antioxidant enzyme activities

To estimate pro-/antioxidant balance in liver mitochondria, we investigated the activity of O_2^- scavenging enzymes Mn-SOD and Cu, Zn-SOD as well as the H_2O_2 removing

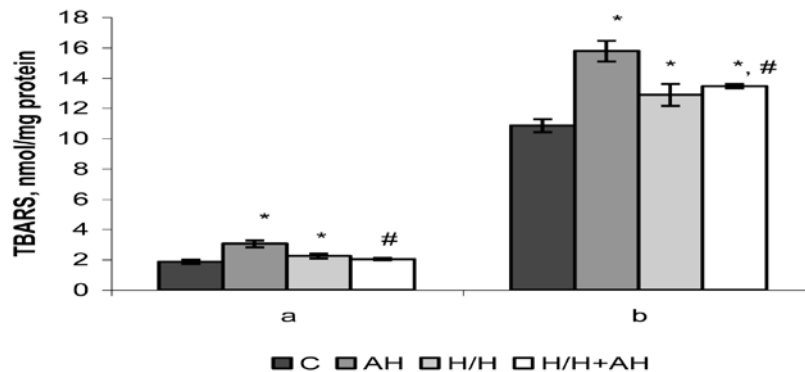


Figure 1. Effect of intermittent hypoxia/hyperoxia and acute hypoxia on mitochondrial lipid peroxidation – basal LPO (a) and Fe^{2+} /ascorbate - induced LPO (b). Values are mean $\pm$ SEM. $n=8$ in each group. The data were analyzed for statistical significance using ANOVA followed by Bonferroni posthoc test. * $P < 0.05$ vs control; # $P < 0.05$ vs acute hypoxia. Groups: C - control; AH- acute hypoxia (7% O_2); H/H - sessions of hypoxia (10% O_2) and hyperoxia (30% O_2) during 14 days; H/H+ AH - acute hypoxia (7% O_2) on the first day after cessation of H/H training.

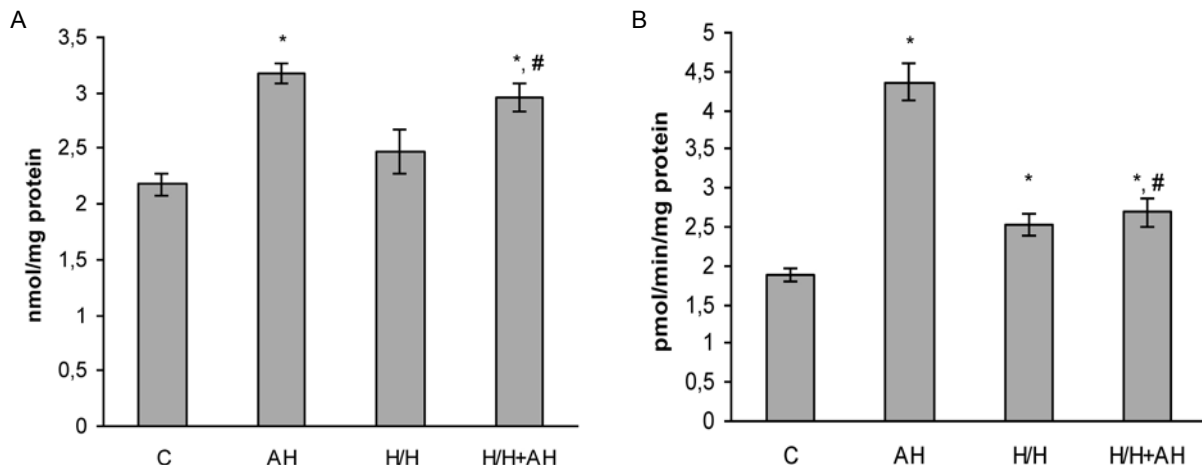


Figure 2. Effect of intermittent hypoxia/hyperoxia and acute hypoxia on mitochondrial protein carbonyls content (A) and H_2O_2 production (B). Values are mean $\pm$ SEM. $n=8$ in each group. The data were analyzed for statistical significance using ANOVA followed by Bonferroni posthoc test. * $P < 0.05$ vs control; # $P < 0.05$ vs acute hypoxia. Groups: C - control; AH- acute hypoxia (7% O_2); H/H - sessions of hypoxia (10% O_2) and hyperoxia (30% O_2) during 14 days; H/H+AH- acute hypoxia (7% O_2) on the first day after cessation of H/H training.

enzyme GPx. Figure 3 illustrates that after acute severe hypoxia, the activity of Mn-SOD was increased by 46% ($P<0.05$) with a concomitant decrease in the activity of Cu, Zn-SOD and GPx by 49% and 23%, respectively, compared to control animals ($P<0.05$). A similar trend in Mn- and Cu, Zn-SOD activities was observed in rats after prolonged H/H (group 3). At the same time, mitochondrial GPx activity was increased by 20% in comparison with control ($P<0.05$). In rats exposed to acute hypoxia after H/H pretreatment, we registered a decrease in mitochondrial Cu, Zn-SOD activity and hyperactivity of Mn-SOD as compared to untreated animals (group 2) ($P<0.05$). However, the GPx activity remained at the control level but was higher than in rats subjected to acute hypoxia alone.

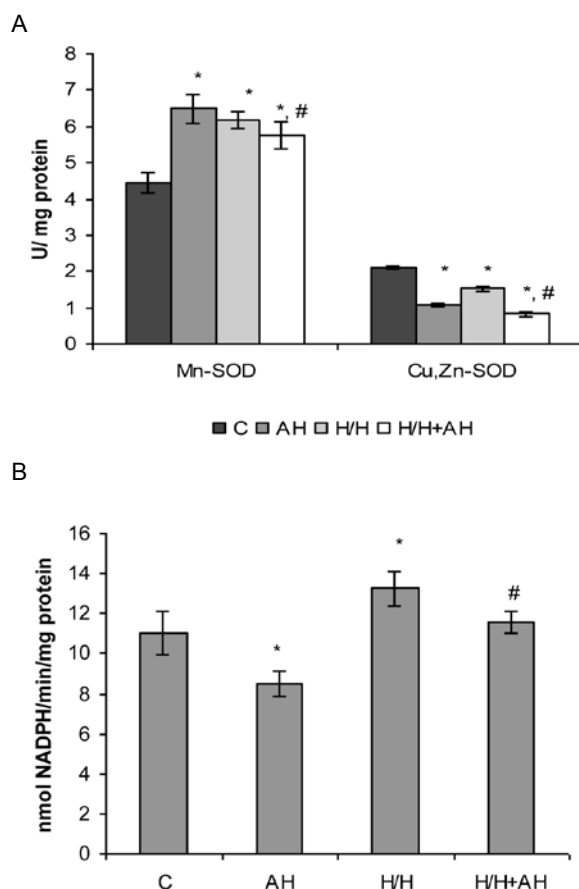


Figure 3. Effect of intermittent hypoxia/hyperoxia and acute hypoxia on activities of Cu,Zn- and Mn-SOD (A) and glutathione peroxidase (B). Values are mean $\pm$ SEM. $n=8$ in each group. The data were analyzed for statistical significance using ANOVA followed by Bonferroni posthoc test. * $P<0.05$ vs control; # $P<0.05$ vs acute hypoxia. Groups: C - control; AH- acute hypoxia (7% O_2); H/H - sessions of hypoxia (10% O_2) and hyperoxia (30% O_2) during 14 days; H/H+AH- acute hypoxia (7% O_2) on the first day after cessation of H/H training.

3.3 Mn-SOD mRNA and protein expression

RT-PCR was performed to identify the differences in Mn-SOD transcripts between control and experimental groups (Figure 4). In order to investigate the translated product of Mn-SOD, Western blot analysis was conducted (Figure 5). Acute hypoxia caused an increase in the MnSOD protein synthesis by 26% ($P<0.05$); no significant change was observed in MnSOD mRNA level in comparison with control. After long-term sessions of H/H (group 3), Mn-SOD protein content had a trend toward decrease, while MnSOD mRNA expression was found to be reduced by 12% as compared to control ($P<0.05$). Acute hypoxia after H/H pretreatment (group 4), increased the MnSOD protein synthesis by 38% ($P<0.05$) without changes in the MnSOD mRNA level.

4. Discussion

In the present study, severe acute hypoxia triggers a series of events including an increase in basal and Fe^{2+} /ascorbate-induced LPO and in H_2O_2 production, as well as an enhance in protein carbonyl content,

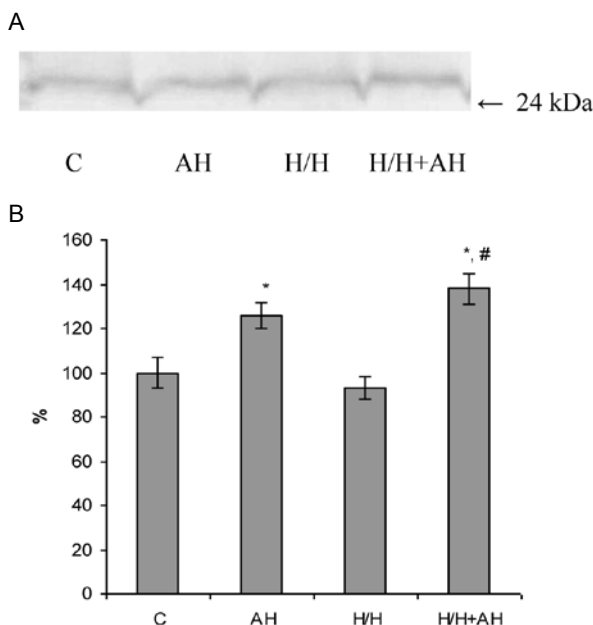


Figure 4. Effect of intermittent hypoxia/hyperoxia and acute hypoxia on Mn-SOD protein expression in liver mitochondria. (A) Representative Western blot and (B) densitometric analysis of Mn-SOD. Isolated mitochondrial protein extracts were separated by performing SDS-PAGE and subsequently electroblotted onto PVDF membranes. The blot was probed using a monoclonal antibody against Mn-SOD. Final Western blot figured as a histogram is expressed as mean percentages ($\pm$ SD) over control values from four independent experiments. Statistically significant differences are indicated as * $P<0.05$ vs. control; # $P<0.05$ vs. acute hypoxia.

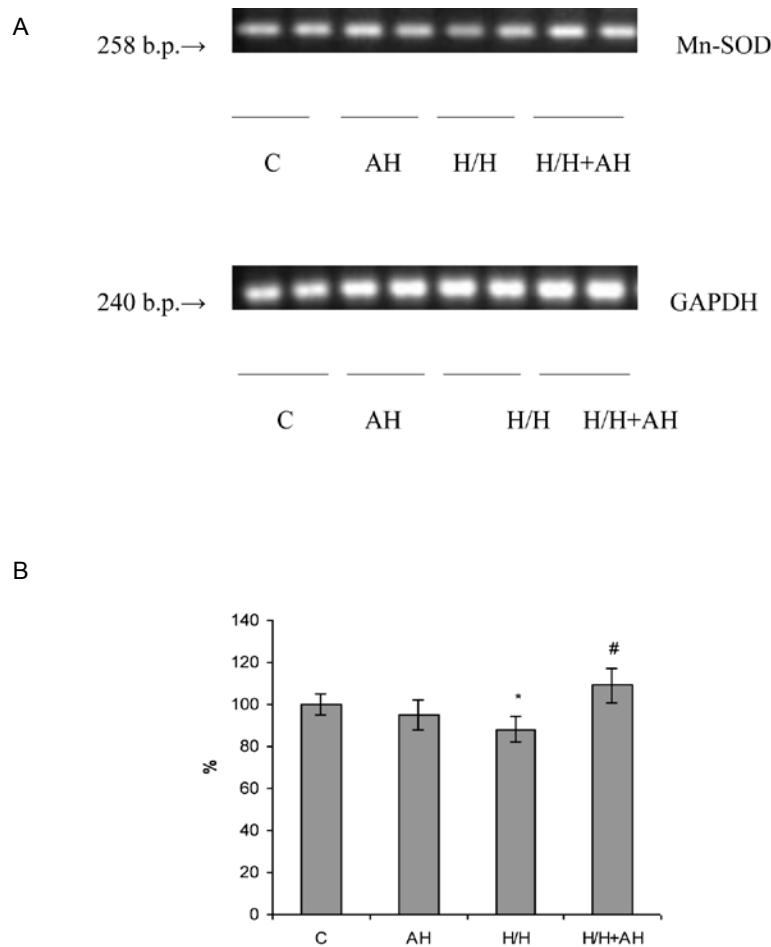


Figure 5. RT-PCR analyses of liver Mn-SOD mRNA levels after intermittent hypoxia/hyperoxia and acute hypoxia (A). Densitometric values corresponding to the levels of Mn-SOD mRNA were normalized to glyceraldehyde-3-phosphatedehydrogenase (GAPDH) mRNA as the internal standard (B). Final data were obtained from four separate experiments and are expressed as mean percentages of control values ($\pm$ SD). Statistically significant differences are indicated as * $P < 0,05$ vs. control; # $P < 0,05$ vs. acute hypoxia.

which served as the biomarkers of general oxidative stress [28]. The oxidative protein damage was provoked by ROS, and RNS and plays a significant role in several pathological conditions such as hypoxia. Radical-mediated protein damage may be initiated by electron leakage, metal-ion dependent reactions, and autooxidation of lipids and sugars [29]. Oxidative modification of proteins are realized via various mechanisms: direct oxidation of amino acid side chains, modification of side chains with lipid peroxidation products (malondialdehyde, acetaldehyde, and 4-hydroxynonenal), or with products of glycation and glycoxidation. All these mechanisms introduce a carbonyl group into the protein that changes its function, increases chemical fragmentation and enhances susceptibility to proteolytic attack [28,30]. Crosslinks of inner mitochondrial membrane proteins by oxidants, or reactive aldehydes generated from lipid peroxidation,

may also result in increased O_2^- and H_2O_2 production, which, in turn, increase the damage and can additionally lead to mitochondrial dysfunction [28].

We found that severe hypoxia significantly altered activities of SOD and GPx - two of the key antioxidant defense enzymes that function in concert to prevent ROS reactions in response to oxidative stress. Overexpression of MnSOD without a concomitant increase in the level of GPx, which we demonstrated in our study, results in the accumulation of H_2O_2 that not only changes the cellular redox status, but also can participate in the Fenton reaction, leading to production of noxious hydroxyl radicals [31]. Our findings suggest that an increase in the LPO level and imbalance in the antioxidant enzyme activity play a pivotal role in mitochondrial dysfunction induced by severe hypoxia insult.

Acute hypoxia in parallel with the peak of ROS generation progressively increased the MnSOD protein

level as well as MnSOD activity, but did not affect the MnSOD mRNA level. At this time point, CuZnSOD activity was repressed, suggesting a different induction of the two SOD isoforms in response to hypoxia [18]. This appears to be in agreement with earlier studies, where immunohistochemical analysis of SODs in the gerbil hippocampus revealed that MnSOD, but not CuZnSOD was increased after short-term ischemia, whereas an overexpression of CuZnSOD could be seen after a longer period of ischemia [32].

In the present study, we showed that the prolonged sessions of short-term hypoxia followed by short-term hyperoxia do not cause changes in protein oxidative modification in the liver mitochondria. However, oxidative changes were registered against the background of an increase in basal and Fe^{2+} /ascorbate-induced LPO as well as in H_2O_2 production.

Superoxide, the first intermediate of free radical processes, is able to initiate free radical chain oxidation, inactivate specific enzymes, and lead to the production of more powerful oxidants by liberation of Fe(II) from the [4Fe-4S] clusters of dehydratases and by reaction with nitric oxide [33].

Sites of mitochondrial ROS generation can be different during hyperoxia and hypoxia. The location of O_2^- within mitochondria is important because O_2^- does not diffuse across mitochondrial membranes. Recent studies suggest that complex I releases O_2^- into the matrix while complex III can release O_2^- into both the matrix and intermembrane space. Exposure to hypoxia may result in the generation of superoxide anion predominantly in the intermembrane space. Such superoxide can be dismutated by Cu,Zn-SOD to H_2O_2 and released into the cytosol. In the cytosol, it may activate the transcription factors, for example, HIF-1. By contrast, exposure to hyperoxia can generate superoxide anion predominantly localized to the matrix. This can explain the observation on isolated cell systems that antioxidant strategies targeting the mitochondrial matrix are more effective than those targeting the cytosol. For example, both in isolated culture and under *in vivo* conditions, the overexpression of Mn-SOD protects against hyperoxia-induced cell death better than overexpression of the Cu,Zn-SOD [4].

After H/H in our model, MnSOD activity was significantly stimulated. This might reflect the mobilization of preexisting enzymes or the result of early SOD gene activation in response to prolonged sessions of hypoxia and hyperoxia. In contrast, mRNA and protein level of MnSOD were found to be reduced. This is consistent with the depletion of energy during hypoxia which influences liver protein synthesis [1,2].

In addition, the occurrence of time lags between induction of SOD mRNA, protein synthesis, and changes in SOD activity under H/H convincingly indicates that the pronounced MnSOD activation is not necessarily accompanied by protein accumulation and can result from the post-translation control, probably from the increased O_2 concentration, which is known to activate the enzyme [34].

Recently, it was reported that MnSOD is an inducible enzyme and that various forms of oxidative stress such as irradiation, hypoxia, hyperoxia, cytokines (IL-1 and -6), interferon- α , and TNF- α influence the MnSOD gene expression [35,36]. Both the relative abundance of mRNA and the catalytic activity of SOD have been shown to correlate with tissue metabolic rate [37]. In our earlier studies, we showed that Mn-SOD is activated in a tissue specific manner [38,39]. It was demonstrated that hypoxia/reoxygenation induce the MnSOD mRNA expression in lung, kidney, and liver tissues [20,36]. Two-week-long exposure to hyperoxia caused an enhancement of the MnSOD immunoreactivity in alveolar type II cells and in interstitial fibroblasts. Under hypoxic condition, MnSOD gene expression can be regulated by redox-sensitive transcription factors, such as SP-1, AP-1, and NF- κB [40]. Some studies demonstrated that the MnSOD-inducing pathway in myocardial preconditioning involves ROS signaling [41] and plays an important protective role against the ischemia/reperfusion injury [19].

In our study, we showed that H/H preconditioning attenuate acute hypoxia-induced ROS generation and protein oxidation in liver mitochondria. In rats adapted to hypoxia/hyperoxia as compared with unadapted animals, we found a decrease in basal and Fe^{2+} /ascorbate-induced LPO, a drop in the hyperactivity of Mn-SOD and, as a consequence, a decrease in H_2O_2 production. We assume that the maintenance of GPx activity on the control level in mitochondria with overexpression of Mn-SOD may suppress acute hypoxia-induced membrane LPO and protein oxidative modification. The latter changes may be assessed as the result of concerted action of SODs and H_2O_2 removing enzymes. In our model, changes in oxygen level caused a decrease in CuZn-SOD activity in the intermembrane space of liver mitochondria. These findings are in agreement with data reported earlier that hypoxia induced no changes in CuZn-SOD mRNA and protein levels [42].

The increase in MnSOD protein synthesis without changes in mRNA level after H/H pretreatment indicates that the MnSOD activity is most likely dependent on the redox-milieu of liver mitochondria, which is related to the balance of MnSOD and H_2O_2 scavenging enzymes. It is assumed that overexpression of MnSOD underlies

the protective/adaptive mechanism against subsequent oxidative stress. A number of studies showed better cell survival under oxidative stress by increasing the level of MnSOD [43,44]. Overexpression of MnSOD protects tissues from ischemia/reperfusion-related damage and increases the resistance of brain membranes to LPO [45], which results in neuroprotection [46]. In a model of myocardial ischemia/reperfusion, MnSOD transgenic mice showed an increase in MnSOD activity, better functional recovery, and limited infarct size [47].

Thus, in this study we demonstrated that prolonged sessions of H/H attenuate basal and Fe²⁺/ascorbate-induced LPO, H₂O₂ production as well as content of carbonyl proteins in liver mitochondria in comparison with untreated rats. The reduction in the intensity of oxidative processes, the induction of MnSOD protein expression and coordinated action of MnSOD and GPx could be the mechanisms underlying the H/H-related protective effect that have contributed to the reduction of acute hypoxia-induced mitochondrial dysfunction.

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