Effects of intermittent hypoxia on oxidative stress-induced myocardial damage in mice

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Park A-M, Suzuki YJ. Effects of intermittent hypoxia on oxidative stress-induced myocardial damage in mice. J Appl Physiol 102: 1806–1814, 2007. First published February 1, 2007; doi:10.1152/japplphysiol.01291.2006.—Obstructive sleep apnea is characterized by episodes of hypoxia and normoxia during sleep, we investigated effects of intermittent hypoxia (IH) on ischemia-reperfusion-induced myocardial injury. C57BL/6 mice were subjected to IH (2 min 6% O2 and 2 min 21% O2) for 8 h/day for 1, 2, or 4 wk; isolated hearts were then subjected to ischemia-reperfusion. IH for 1 or 2 wk significantly enhanced ischemia-reperfusion-induced myocardial injury. However, enhanced cardiac damage was not seen in mice treated with 4 wk of IH, suggesting that the heart has adapted to chronic IH. Ischemia-reperfusion-induced lipid peroxidation and protein carbonylation were enhanced with 2 wk of IH, while, with 4 wk, oxidative stress was normalized to levels in animals without IH. H2O2 scavenging activity in adapted hearts was of IH, while, with 4 wk, oxidative stress was normalized to levels in ischemia-reperfusion-induced myocardial injury. Ischemia-reperfusion-induced oxidative stress was enhanced with 2 wk of IH, yet, with 4 wk, oxidative stress was normalized to levels in animals without IH. H2O2 scavenging activity in adapted hearts was higher after ischemia-reperfusion, suggesting the increased antioxidant capacity. This might be due to the involvement of thioredoxin, as the expression level of this protein was increased, while levels of other antioxidant enzymes were unchanged. In the heart from mice treated with 2 wk of IH, ischemia-reperfusion was found to decrease thioredoxin. Ischemia-reperfusion injury can also be enhanced when thioredoxin reductase was inhibited in control hearts. These results demonstrate that IH changes the susceptibility of the heart to oxidative stress in part via alteration of thioredoxin.

Ischemia; obstructive sleep apnea; oxidative stress; thioredoxin

OBSTRUCTIVE SLEEP APNEA (OSA) syndrome is a condition characterized by the occurrence of repetitive episodes of airflow obstruction during sleep. OSA is a common disorder in the United States and other Western countries, with the prevalence reported to be 4% in men and 2% in women (46). Besides the obvious detrimental effect of OSA, such as daytime sleepiness, deficits in cognitive performance, and mood and behavioral effects, this syndrome is also associated with an increased risk for cardiovascular diseases. OSA has been implicated in pathogenesis of systemic hypertension, pulmonary hypertension, congestive cardiac failure, cardiac arrhythmias, atherosclerosis, stroke, and ischemic heart disease (3, 12, 24, 29, 31, 33, 35–38). Among various possible mechanisms, oxidative stress may play roles in pathogenesis of OSA-associated disorders (41).

Ischemic injury to myocardium is the leading cause of death in the US and other Western countries. Postischemic dysfunction persists after reperfusion, despite the absence of irreversible damage and a restoration of normal coronary flow (6), and this may be mediated by the generation of reactive oxygen species (ROS). Evidence for the role of ROS in ischemia-reperfusion (I/R) injury was obtained in experiments in which the administration of antioxidants was shown to enhance the recovery of functions after reperfusion (15, 22, 27, 34).

Periodic obstruction of the upper airway leads to decreased blood oxygenation and intermittent hypoxia (IH) that is characterized by repeated episodes of hypoxia/reoxygenation. Recent reports show that IH can have profound effects on I/R injury; in some cases, IH was found to elicit a preconditioning-like event (8, 10, 11), while in others, chronic IH increased the susceptibility to I/R injury (17). As repeated episodes of brief ischemia and reperfusion can serve as a powerful mechanism for protection against subsequent lethal I/R injury, mild stress might promote cardioprotective mechanisms. Thus short IH might enhance defense mechanisms to cope with subsequent strenuous stress, while extended IH beyond protective periods might turn to a condition with increased susceptibility to oxidative stress.

The present study reports that the heart also has the ability to adapt to the condition of the IH-mediated enhancement of the susceptibility to I/R. We found that a treatment of mice with IH for 1–2 wk increased the susceptibility of the heart to I/R injury. However, after 4 wk of IH, the susceptibility of the heart to I/R was normalized to the level similar to animals without IH treatment. Promotion of oxidative stress appears to be involved in the increased susceptibility to I/R injury in mice treated with 1–2 wk of IH. We provide evidence that the mechanism of IH modulation of the susceptibility of the heart to I/R involves a redox regulator, thioredoxin.

MATERIALS AND METHODS

Animals. Male C57BL/6 mice (8–11 wk old) were placed in a chamber for the OxyCycler Oxygen Profile Controller (BioSpherix, Redfield, NY) that was set to cycle between 6 and 21% O2 every 2 min continuously for 8 h/day (10 AM to 6 PM) during the normal sleeping period of mice for 1, 2, or 4 wk. Normoxia controls were subjected to ambient 21% O2 in the separate OxyCycler chamber. Systolic, diastolic, and mean blood pressure, and heart rate were monitored using the Coda 6 Noninvasive Blood Pressure System (Kent Scientific, Torrington, CT) with two separate cuffs: the first positioned closely to the tail base and used for arterial occlusion, and the second positioned down from the first cuff for sensing arterial pulsation in the morning before mice underwent IH treatment. Animals were fed normal rat chow, and all protocols involving animals were approved by the Georgetown University Animal Care and Use Committee and abide by the National Institutes of Health guidelines.

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Isolated heart perfusion studies. Hearts were rapidly removed from mice anesthetized with inhalation of isoflurane. The aorta was cannulated with a cannula connected to the Langendorff apparatus. The Langendorff perfusion was initiated instantly after heart excision with modified Krebs-Henseleit (KH) buffer, containing (in mM) 118.0 NaCl, 4.7 KCl, 1.7 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.0 NaHCO₃, and 10.0 glucose. Buffer was continuously bubbled with 95% O₂ and 5% CO₂ (pH 7.4, 37°C) throughout the perfusion period. After 15 min of stabilization period, the hearts were subjected to a 30-min no-flow ischemia followed by 180 min of reperfusion.

For treatment of the heart with an irreversible thioredoxin reductase inhibitor, 1-chloro-2,4-dinitrobenzene (DNCB), hearts were isolated and subjected to Langendorff perfusion. After 15-min equilibration, hearts were perfused with or without 50 μM DNCB in KH buffer for 30 min. Hearts were then subjected to 30-min global ischemia followed by 180-min reperfusion.

Lactic dehydrogenase leakage. Coronary effluent was collected from the heart at 5 min before ischemia, as well as 1, 5, 10, 15, 20, 25, and 30 min during the reperfusion period. The effluent was mixed with 0.1 M glycine-NaOH buffer (pH 10), 25 mM Na-DL-lactate, and 1 mM NAD, and the absorbance of 340 nm was continuously measured for 5 min using spectra max 340PC (Molecular Devices, Sunnyvale, CA).

Measurements of myocardial infarction. At the end of each experiment, the heart was immediately removed from the Langendorff apparatus. The heart was frozen and cut into 1.0-mm-thick slices. The

![Fig. 1. Effects of intermittent hypoxia (IH) on ischemia-reperfusion (I/R)-induced myocardial injury.](http://jap.physiology.org/)

A: effluents were collected from the hearts, and the lactic dehydrogenase (LDH) activity was measured by spectrometric method. The line graph represents means ± SE of time course of LDH leakage detected in effluents. The bar graph shows means ± SE of LDH leakage at 1 min after reperfusion. Statistically significant differences were determined by one-way ANOVA (effect of IH, P < 0.001). *P < 0.05.

B: the heart slices were incubated with 2,3,5-triphenyltetrazolium chloride (TTC), and myocardial infarction was monitored. Representative sections are shown on the left, and the bar graph represents means ± SE (n = 4) of percentage of area with infarction after 180 min of reperfusion. Statistically significant differences were determined by two-way ANOVA (effect of IH, P = 0.0072 and I/R, P < 0.0001). *Values significantly different from each other at P < 0.05 as determined by post hoc test. cont, Control.

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### Table 1. Effects of intermittent hypoxia on body weight

<table>
<thead>
<tr>
<th>n</th>
<th>Body Weight, g</th>
<th>SEM</th>
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<tbody>
<tr>
<td>19</td>
<td>26.1 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>24.0 ± 0.21*</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>24.5 ± 0.28*</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>24.7 ± 0.31*</td>
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</tr>
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</table>

Values represent means ± SE; n, no. of animals. Statistically significant differences were determined by one-way ANOVA (effect of intermittent hypoxia, P < 0.001). *P < 0.05.

### Table 2. Effects of intermittent hypoxia on blood pressure and heart rate

<table>
<thead>
<tr>
<th>n</th>
<th>Systolic BP, mmHg</th>
<th>Mean BP, mmHg</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>136.6 ± 4.5</td>
<td>124.8 ± 5.3</td>
<td>660.2 ± 25.1</td>
</tr>
<tr>
<td>1 wk</td>
<td>150.5 ± 4.7*</td>
<td>142.8 ± 6.5</td>
<td>680.2 ± 35.2</td>
</tr>
<tr>
<td>2 wk</td>
<td>161.3 ± 3.8*</td>
<td>153.7 ± 3.8*</td>
<td>576.7 ± 48.2</td>
</tr>
<tr>
<td>4 wk</td>
<td>154.4 ± 4.5*</td>
<td>144.9 ± 4.8*</td>
<td>669.3 ± 40.1</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n, no. of animals. Statistically significant differences were determined by one-way ANOVA (effect of intermittent hypoxia, P < 0.05). *P < 0.05.
slices were incubated in 1% 2,3,5-triphenyltetrazolium chloride in PBS (pH 7.4) for 30 min at 37°C. Stained slices were fixed in buffered paraformaldehyde for 10 min and then placed between glass plates. Percentage of the infarcted (white) area relative to the total area was quantified using the ImageJ software (National Institutes of Health).

RT-PCR. Total RNA (1 μg) extracted from hearts using TRIzol (Invitrogen, Carlsbad, CA) was reverse transcribed by oligo(dT) priming and Moloney murine leukemia virus reverse transcriptase (Applied Biosystems, Foster City, CA). The resultant cDNA was amplified using Taq DNA polymerase (Invitrogen) and resolved on a 1.5 or 2% agarose gel containing ethidium bromide. PCR primers for mouse gata4 were used as previously described (19). PCR primers for mouse bcl-xL are as follows: 5′ primer, 5′-CAT CCA AAC TGC TGC TGT GG-3′, and 3′ primer, 5′-TTA TCT TGG CTT TGG ATC CTG-3′, which produce a 337-base pair product. PCR primers for mouse thioredoxin-1 are as follows: 5′ primer, 5′-CGT GGT GGA CTT CTC TGC TAC GTG GTG-3′ and 3′ primer, 5′-CTG CAT GCA TTT GAC TTC ACA GTC-3′, which produce a 160-base pair product. Denaturing was performed at 94°C for 45 s, annealing for 45 s at 53°C (for bcl-xL) or 58°C (for thioredoxin-1), and polymerase reactions for 2 min at 72°C (30 cycles). The g3pdh mRNA level was also monitored using primers from BD Biosciences Clontech (Palo Alto, CA) as an internal control.

EMSA. Heart ventricles were homogenized by Polytron (Kinematica, Switzerland) in four volumes of ice-cold homogenization buffer containing 10 mM HEPES (pH 7.5), 0.5 M sucrose, 0.05 M spermine, 0.15 mM spermin, 2 mM EDTA, 2 mM EGTA, 1 mM sodium fluoride, 0.1 mM sodium orthovanadate, 1 mM tetrasodium pyrophosphate, 1 mM PMSF, 5 μg/ml leupeptin, and 10 μg/ml aprotinin. Nuclear-rich fractions were prepared by the following procedures (28). Heart homogenates were centrifuged at 12,000 g for 30 min at 4°C. Pellets were lysed in two volumes of ice-cold homogenization buffer containing 0.1% Igepal and 0.5 M sucrose by homogenizing with Polytron, then centrifuged at 12,000 g for 30 min at 4°C, and washed with ice-cold homogenization buffer containing 0.35 M sucrose. After being washed, the nucleus was extracted with one volume of ice-cold homogenization buffer containing 0.3 M NaCl and 10% glycerol for 60 min at 4°C with mixing at 1,400 rpm. Nuclear extracts were collected by centrifugation at 16,000 g for 30 min at 4°C.

For EMSA, the binding reactions were performed for 20 min in 5 mM TrisHCl (pH 7.5), 37.5 mM KCl, 4% (wt/vol) Ficoll 400, 0.2 mM EDTA, and 0.5 mM spermine, and total protein concentration was 3 μg/μl for each sample. No significant differences were observed between samples. Oligonucleotide probe containing the proximal GATA binding element in the bcl-xL promoter. SS denotes a supershift experiment with the GATA-4 antibody and control nuclear extracts with an arrow indicating the supershifted band. Free probe (FP) and FP+Ab denote the oligonucleotide without nuclear extracts and the oligonucleotide with GATA-4 antibody without nuclear extract, respectively. The line graph shows means ± SE (n = 4) of the fold increase relative to normoxic controls. *Values significant different from each other at P < 0.05 as determined by post hoc test.
mM EDTA, 0.5 mM DTT, 1 μg poly(dI-dC)-poly(dI-dC), 0.25 ng (>20,000 cpm) 32P-labeled double-stranded oligonucleotide, and 10 μg protein of nuclear extract. Electrophoresis of samples through a native 6% polyacrylamide gel was followed by autoradiography. The double-stranded oligonucleotide probes used in this study are the proximal GATA element from the bcl-xL promoter with a sequence, 5'-AAG CCA AGA TAA GGT TCT (1). Supershift (SS) experiments were performed by incubating nuclear extracts with 2 μg of GATA-4 antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

**Western blot analysis.** To prepare total tissue homogenates, heart ventricles were solubilized with 50 mM HEPES solution (pH 7.4) containing 1% (vol/vol) Triton X-100, 4 mM EDTA, 1 mM sodium fluoride, 0.1 mM sodium orthovanadate, 1 mM tetrasodium pyrophosphate, 2 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. Equal protein amounts (10 μg) of heart homogenates were electrophoresed through a reducing SDS polyacrylamide gel and electrobotted onto a nitrocellulose membrane. The membrane was blocked and incubated with the polyclonal IgG for catalase (Bioblots, Darmstadt, Germany), Cu,Zn-superoxide dismutase, Mn-superoxide dismutase, glutathione peroxidase, or thioredoxin-1 (Santa Cruz Biotechnology), and the detection was made with horseradish peroxidase-linked secondary antibodies and ECL System (Amersham Life Science, Arlington Heights, IL). Membranes were then stained with Coomassie Brilliant Blue R-250 to be used as loading controls.

**Measurements of protein carbonylation.** To monitor carbonylated proteins, heart homogenate proteins were derivatized with dinitrophenylhydrazine, then subjected to SDS-PAGE (10% acrylamide gel). Proteins were transferred on a nitrocellulose membrane, and carbonylated proteins were visualized using OxyBlot Protein Oxidation Detection Kit (Chemicon International, Temecula, CA) and immunoblot. The detection was made with horseradish peroxidase-linked secondary antibodies and ECL System.

**Measurements of lipid peroxidation.** As an index of oxidative damage, lipid peroxidation was evaluated by monitoring the levels of thiobarbituric acid-reactive substances (TBARS) (7). Heart homogenates were mixed with 0.125 ml of a solution containing 26 mM thiobarbituric acid, 0.26 M HCl, 15% trichloric acid, and 0.02% butylated hydroxytoluene. The mixtures were heated at 96°C for 15 min and then centrifuged at 8,000 rpm for 5 min. The supernatant was transferred to a microplate, and the absorption was measured at 532 nm. Concentrations of malonaldehyde were calculated from the absorption values using tetramethoxy propane as standard.

**Measurement of H2O2 scavenging activity.** The hydrogen peroxide (H2O2) scavenging activity in heart homogenates was measured spectrophotometrically by following the decrease in the H2O2 concentration over 60 s (linear least squares fittings) at 240 nm (ε240 = 43.6 M-1 cm-1, where ε is molar extinction coefficient) (4). The absorbance at 240 nm was continuously measured for 3 min by a spectrometer.

**Statistical analysis.** Means ± SE were calculated, and ANOVA and post hoc test with Bonferroni method for multiple comparisons were used for statistical analyses. Statistically significant differences between two groups were determined by the Student’s t-test at P < 0.05.

**RESULTS**

**Effects of IH on cardiovascular parameters.** Male C57BL/6 mice were subjected to episodic hypoxia (2 min of 6% O2 and 2 min of 21% O2) for 8 h/day for 1, 2, or 4 wk. A slight decrease (<8%) in total body weight was noted after 1 wk of IH (Table 1). Consistent with OSA patients developing systemic hypertension (29, 33), IH increased mean arterial blood pressure and systolic blood pressure (Table 2). No significant changes in heart rate were noted (Table 2).

**Effects of IH on myocardial I/R injury.** As IH involves hypoxia/exposure cycles, which could produce oxidative stress, we hypothesized that treatment of mice with chronic IH might exaggerate myocardial I/R injury. Mice were subjected to IH cycles of 2 min hypoxia (6% O2) and 2 min normoxia for 1, 2, or 4 wk. After IH treatment, hearts were excised and subjected to Langendorff perfusion to induce 30-min global ischemia followed by reperfusion.

The leakage of lactic dehydrogenase (LDH) from the heart is often used as a measure of cardiac damage in response to I/R...
Injury. As shown in Fig. 1A, effluent LDH levels increased from 0 before ischemia to \(\sim 200 \text{ mU/ml} \) 1 min after reperfusion. The I/R-induced LDH leakage was substantially higher in hearts from mice subjected to IH for 1 wk (>400 mU/ml) or 2 wk (>600 mU/ml). Surprisingly, however, I/R-induced LDH leakage was similar to (or even less than) controls in hearts of mice subjected to 4 wk of IH. IH did not alter basal LDH levels in effluent before ischemia.

I/R also caused myocardial infarction as monitored by 2,3,5-triphenyltetrazolium chloride assay. The degree of myocardial infarction induced by I/R was dramatically increased in hearts from mice that were exposed to IH for 1 or 2 wk (Fig. 1B). However, like the measurement of LDH leakage, hearts from mice that were exposed to 4 wk of IH had I/R-induced myocardial infarction levels similar to control hearts from normoxic mice.

These data suggest that 1–2 wk of IH results in increased susceptibility of the heart to I/R injury, while the heart appears to adapt to IH by 4 wk by normalizing its susceptibility to I/R injury.

Effects of IH on bcl-xL and GATA-4. We found that 1–2 wk of IH, which causes exaggerated I/R injury, was associated with increases in stress response factors, such as bcl-xL, which may attempt to prevent apoptosis (Fig. 2A). Interestingly, consistent with the results of myocardial infarction and LDH leakage, the bcl-xL expression was normalized to

Fig. 4. Effects of IH on antioxidants. Mice were subjected to IH for 1, 2, or 4 wk. Hearts were isolated and subjected to Langendorff perfusion to elicit 30-min global ischemia followed by reperfusion. A: hydrogen peroxide (H\(_2\)O\(_2\)) scavenging activities in ventricular homogenates were measured spectrophotometrically by following the decrease of the H\(_2\)O\(_2\) concentration at 240 nm over 60 s. The bar graphs represent means ± SE (n = 4). Statistically significant differences were determined by two-way ANOVA (effect of I/R, \(P = 0.045\) and no effect of IH). Western blot was used to monitor protein expression levels of catalase (B), glutathione peroxidase (GPx; C), Cu, Zn-superoxide dismutase (Cu, Zn-SOD; D), and Mn-superoxide dismutase (Mn-SOD; E). The bar graphs represent means ± SE (n = 4) of fold increase in enzyme expression relative to control. *Values significantly different from each other at \(P < 0.05\) as determined by post hoc test.
control levels in the hearts from mice treated with 4 wk of IH.

A key regulator of bel-xL gene transcription and a novel stress-responsive transcription factor, GATA-4 (1, 21, 39, 40), was also found to be similarly affected by IH. The mRNA level of this transcription factor was found to be increased with 1 or 2 wk of IH (Fig. 2B). With 4 wk of IH, the level of gata4 mRNA returned to normal (Fig. 2B).

Consequently, the DNA binding activity toward the proximal GATA element within the bel-xL gene promoter was increased at 1 and 2 wk of IH, while the binding activity in the hearts of mice exposed to 4 wk of IH was comparable to that of the controls (Fig. 2C). SS experiments revealed that the GATA binding activity in mouse hearts is largely due to GATA-4 (Fig. 2C).

These results further demonstrate that the hearts from mice treated with 1–2 wk of IH are in stress, while those from animals subjected to 4 wk of IH have normalized stress-responsive gene expression.

Effects of IH on oxidative stress. As I/R injury has been shown to involve oxidative stress (6, 15, 22, 27, 34), we measured indications of lipid peroxidation and protein oxidation 3 h after the onset of reperfusion subsequent to ischemia. Measurements of protein carbonyl content showed that protein oxidation induced by I/R is significantly increased at 2 wk of IH, while, at 4 wk, the levels of protein oxidation were normalized to the control level (Fig. 3A). Similar results were obtained when we measured TBARS levels as an indication of lipid peroxidation (Fig. 3B).

The mechanism of reduced oxidative stress in the hearts of mice treated with 4 wk of IH might be explained by the observations that I/R increases the H2O2 scavenging activity in these hearts (Fig. 4A). However, protein levels of neither of the major H2O2 scavenging enzymes, catalase (Fig. 4B) nor glutathione peroxidase (Fig. 4C), were altered after I/R in adapted hearts. The levels of superoxide dismutases were also unchanged (Fig. 4, D and E).

We found that thioredoxin, which can scavenge H2O2, is upregulated in adapted hearts in response to I/R. As shown in Fig. 5A, the level of thioredoxin protein was significantly higher after I/R compared with before I/R in adapted hearts from mice subjected to 4 wk of IH. Interestingly, hearts from mice treated with 2 wk of IH with increased susceptibility to I/R had a reduced thioredoxin-1 level after I/R. mRNA levels of thioredoxin-1 also exhibited similar trends (Fig. 5B).

To provide direct evidence that thioredoxin can regulate I/R-mediated cardiac damage, we studied the effects of an irreversible thioredoxin reductase inhibitor, DNCB (2), in per-
fused isolated mouse hearts. We found that DNCB significantly enhanced I/R-induced LDH leakage (Fig. 6A; solid square vs. solid circle). DNCB also enhanced myocardial infarction (Fig. 6B) and protein carbonylation (Fig. 6C) induced by I/R. These results provide evidence that inhibition of the thioredoxin system can increase myocardial damage induced by I/R, similar to the observations in hearts from mice treated with 2 wk of IH.

DISCUSSION

The major finding of this study is that the heart increases its susceptibility to I/R injury when mice are treated with IH for 1 or 2 wk, but the susceptibility to I/R stress is normalized with 4 wk of IH. Understanding the mechanisms of the influence of different stages of IH on oxidative stress-mediated myocardial damage should help reveal pathogenic mechanisms associated with OSA. We present evidence that a redox regulator, thioredoxin, plays a role in this adaptive mechanism.

OSA has been shown to be associated with myocardial ischemia (9, 13, 25, 32, 42). Apnea index is an independent predictor of myocardial infarction after adjusting for age, body mass index, hypertension, smoking, and cholesterol level (16). Thus the increased ischemic myocardial damage in mice treated with chronic IH, as shown in the present study, as well

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**Fig. 6. Effects of 1-chloro-2,4-dinitrobenzene (DNCB) on I/R-induced heart damage.** Mouse hearts were isolated and subjected to Langendorff perfusion. After 15-min equilibration, heart were perfused with or without 50 μM DNCB in Krebs-Henseleit buffer for 30 min. Then hearts were subjected to 30-min global ischemia followed by 180-min reperfusion. A: effluents were collected from the hearts. The LDH activity was measured by a spectrometric method. The line graph represents means ± SE of time course of LDH leakage detected in effluents. Data on hearts from mice treated with 2 wk of IH are also shown. *Values significantly different between control + I/R and DNCB + I/R groups as well as control + I/R and IH + I/R groups at all points from time 35–55 min at *P* < 0.05 (n = 4). B: the heart slices were incubated with TTC. The bar graph represents means ± SE (n = 4) of percentage of area with infarction. Representative sections are shown at the bottom. C: levels of carbonylated proteins in heart homogenates were determined by OxyBlot Protein Oxidation Detection Kit. Values represent means ± SE of fold increase in total carbonyl content as determined by densitometry. *Values significantly different from each other at *P* < 0.05 as determined by the Student’s *t*-test (n = 4).
as in rats previously reported by Joyeux-Faure et al. (17), might resemble the conditions of OSA patients. However, previous reports on the observations that IH can promote cardioprotection (5, 8, 30) and the finding in the present study that the heart can adapt to IH complicate the interpretations of the cardiac effects of IH in relation to clinical relevance. One explanation is that up to 4 wk of IH might represent initial responses of the body to oxygen tension changes. Longer periods of IH treatment beyond the adaptation phase might result in sustained enhancement of the heart to I/R injury. Another possibility is that protective and adaptive mechanisms might actually represent important events, which might occur in OSA patients. It has been reported that, in elderly people, OA may have protective effects against mortality (23). It should also be noted that, while many OSA patients are obese, mice treated with chronic IH had reduced body weight. Thus obesity and metabolic alterations might worsen cardiovascular functions in OSA patients. Understanding of complex actions of IH, which can exert both protective and detrimental effects, might reveal important information for developing therapeutic strategies for better managing OSA patients.

The leakage of LDH and other proteins occurs after I/R due to disturbed membrane structure, which might be due to the promotion of lipid peroxidation (43). Consistently, the degree of I/R-induced protein oxidation as assessed by measuring protein carbonyl content was enhanced by 1 or 2 wk of IH, indicating the increased capacity to promote oxidative stress in susceptible hearts. Similarly, the levels of lipid peroxidation as monitored by detecting TBARS formation was also enhanced in response to I/R in susceptible hearts. In contrast, the hearts from mice treated with 4 wk of IH did not have exaggerated oxidative stress in response to I/R, suggesting that the adaptation mechanism may involve either the reduction of ROS production or increased antioxidant capacity. Interestingly, we found that the ability of the heart homogenates to scavenge H2O2 was increased after I/R in the adapted hearts. We measured the levels of known H2O2 scavenging enzymes, i.e., catalase and glutathione peroxidase; however, these were not increased in accord with the increased H2O2 scavenging activity. We provide evidence that this H2O2 scavenging activity is dependent on thioredoxin.

Thioredoxin is a ubiquitous protein with redox-active cysteine residues. The reduced thioredoxin acts as an electron donor and also scavenges intracellular H2O2 while being catalyzed by a family of thioredoxin-dependent peroxidases, peroxiredoxins (14). The cardioprotective roles of thioredoxin have been demonstrated in transgenic mice overexpressing thioredoxin-1 (44). Hearts from thioredoxin-1 overexpressing transgenic mice display significantly improved postischemic ventricular recovery and reduced myocardial infarct size and apoptosis, compared with wild-type mouse hearts. In the present study, the myocardial expression of this redox molecule was found to be increased after I/R in the adapted hearts. mRNA levels of thioredoxin-1 were also increased. The thioredoxin gene has been shown to be regulated by antioxidant response element with binding of transcription factors such as NF-κB and Maf, and Jun/Fos (18). These molecules may regulate the increase of thioredoxin-1 mRNA in the adapted hearts. Our results showing that thioredoxin reductase inhibitor, DNCB, enhances I/R-induced injury support the hypothesis that thioredoxin is involved in protecting the heart. The oral administration of another thioredoxin reductase inhibitor, aurinorfin, has also been shown to impair the recovery of the heart from I/R injury (45). Since thioredoxin is downregulated in response to I/R in the hearts of mice treated with 2 wk of IH, inhibition of thioredoxin system may also play a role in the enhanced oxidative stress-mediated myocardial damage in response to chronic IH.

Cell survival factors such as bcl-xL are often increased in response to stress. Consistently, bcl-xL mRNA expression is enhanced at 1 and 2 wk of IH when the heart is more susceptible to I/R stress. As adaptation occurs with 4 wk of IH, such stress responses diminished. The bcl-xL expression has been shown to be regulated by the GATA-4 transcription factor in the heart (1, 21). We found that, as bcl-xL mRNA expression is increased, the DNA binding activity of GATA-4 toward the proximal GATA binding element of the bcl-xL promoter is enhanced. While GATA-4 activation has been reported to occur mainly through posttranslational modification mechanisms (20, 26, 40), we found that gata-4 mRNA levels were increased at 1 and 2 wk of IH when the GATA-4 DNA binding activity was enhanced. Similarly, when the GATA-4 DNA binding activity returned to the control level at 4 wk of IH, the gata-4 mRNA level also decreased to the control level.

In summary, we found a novel mechanism of IH to influence the susceptibility of the heart to I/R injury. Along with abilities of IH to promote preconditioning-like effects and to enhance the susceptibility of the heart to I/R injury, an adaptation mechanism serves as another event by which IH, and perhaps OSA, might influence the heart. We propose that thioredoxin plays roles in IH-mediated alterations of the susceptibility of the heart to oxidative stress. Understanding the complex mechanisms for IH-mediated cardiac alterations should help developing therapeutic strategies to prevent and/or treat OSA-induced cardiovascular complications.

GRANTS

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