Role of Sodium-Hydrogen Exchanger-1 (NHE-1) in the Effect of Exercise on Intermittent Hypoxia-Induced Left Ventricular Dysfunction

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Abstract

Intermittent hypoxia (IH) occurs frequently in patients with obstructive sleep apnoea and can cause ventricular dysfunction. However, whether myocardial inflammation and sodium-hydrogen exchanger-1 (NHE-1) expression play an important role in IH-induced ventricular dysfunction remains unclear. This study aimed to investigate whether short-term exercise provides a protective effect on IH-induced left ventricular (LV) function impairment. Male Sprague-Dawley rats were randomly assigned to 4 groups: control (CON), IH, exercise (EXE) or IH interspersed with EXE (IHEXE). IH rats were exposed to repetitive hypoxia/reoxygenation cycles (2%-6% O_2 for 2-5 s per 75 s, followed by 21% O_2 for 6 h/day) during the light phase for 12 consecutive days. EXE rats were habituated to treadmill running for 5 days, permitted 2 days of rest, and followed by 5 exercise bouts (30 m/min for 60 min on a 2% grade) on consecutive days during the dark phase. IHEXE rats were exposed to IH during the light phase interspersed with exercise programs during the dark phase on the same day. Cardiac function was quantified by echocardiographic evaluation. Myocardial levels of tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and NHE-1 were determined. IH rats showed LV dysfunction characterized by lower LV fractional shortening (LVFS%) and LV ejection fraction (LVEF%). LV dysfunction was associated with higher myocardial levels of TNF-α, IL-6 and NHE-1 mRNA and protein. These changes were not observed in IHEXE rats ($P > 0.05$ for all). EXE rats showed lower levels of NHE-1 protein than CON rats ($P < 0.05$). However, the levels of LVFS%, LVEF%, TNF-α and IL-6 protein and NHE-1 mRNA did not differ between EXE and CON rats ($P > 0.05$ for all). These data indicated that exercise may provide a protective effect on IH-induced LV dysfunction by attenuating IH-induced myocardial NHE-1 hyperactivity.

Key Words: cardioprotection, exercise, inflammation, intermittent hypoxia, left ventricular, sodium-hydrogen exchanger-1

Introduction

Obstructive sleep apnoea (OSA) is associated with cardiovascular morbidity and mortality largely as a result of myocardial dysfunction (1). OSA remains a serious public health problem in the developed world because its prevalence among the general population ranges from 2% to 33% (22). The major factor contributing to OSA-related myocardial damage is intermittent hypoxia (IH), resulting from recurrent episodes of reduction and restoration of arterial oxygen saturation (10) during periodic obstruction and ventilation resumption of the upper airway in patients with OSA (6, 9, 29).

There are accumulating evidences that IH plays a role in the pathogenesis of cardiovascular complications in OSA through activation of pro-inflammatory pathways (17, 38, 42, 43). The levels of circulating pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) (42), soluble adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), and chemokines such as monocyte chemoattractant protein-1 (MCP-1) are found to be elevated in patients with OSA (24). Increased monocyte infiltration with increased MCP-1 gene expression and serum concentration of MCP-1 is observed in the myocardium of animals and patients with heart failure (HF). MCP-1 overexpression in the myocardium can have a negative effect on left ventricular (LV) remodelling and subsequently worsen HF (28). However, it remains unknown how IH affects myocardial inflammatory cytokines production in IH-induced LV dysfunction.

Sodium-hydrogen exchanger-1 (NHE-1) is an essential regulator of pH, and activation of NHE-1 has been implicated as a critical element of ischemia/reperfusion (16). Intracellular protons are exchanged for extracellular Na^+ *via* NHE-1, and the rising intracellular Na^+ can produce an increase in intracellular Ca^{2+}, contributing to the generation of IL-6 and TNF-α following nuclear transcription factor-κB translocation (3, 25). NHE-1 up-regulation has been reported in the pathogenesis of HF (12), and NHE inhibition has been demonstrated in numerous animal and human studies to improve cardiac function during ischemia/reperfusion. It can also attenuate myocardial inflammation by reducing neutrophil accumulation in risk areas (2). However, whether IH can induce NHE-1 activation in the myocardium remains unclear.

Recently, epidemiological investigations have indicated that regular exercise can have positive influences on patients with OSA. Exercise can reduce cardiac sympathetic over-activity and increase peak maximal oxygen uptake in patients with sleep disorders (36, 39, 40). In addition, an animal study demonstrated that exercising at a certain intensity could result in cardiac hypertrophy and the improved cardiac performance was associated with decreased myocardial NHE-1 levels (16). However, it remains unclear whether exercise plays a role in NHE inhibition and reduces myocardial inflammation in patients with OSA. This study aimed to investigate whether exercise causes NHE-1 inhibition and reduces myocardial inflammation that contributes to improving IH-induced LV dysfunction in an IH rat model mimicking IH/reoxygenation of OSA.

Materials and Methods

Animal Preparation

Experiments were performed on male Sprague-Dawley rats weighing 300-350 g. Nine week-old rats were maintained on an artificial 12-h light-dark cycle. The light period began at 0700 h. Rats were randomly assigned to 4 groups: control (CON), IH, exercise (EXE) or IH interspersed with EXE (IHEXE) (n = 10 per group). CON rats were handled similarly to IH, EXE and IHEXE rats but did not undergo any experimental protocols. IHEXE rats were exposed to IH during the light phase interspersed with exercise during the dark phase on the same day. Water and food were available *ad libitum*. No significant differences in initial age or body weight were found between the groups (Table 1). Rat body weight was measured daily at 0900 h. All surgical and experimental procedures were conducted using recommended procedures approved by the Institutional Animal Care and Use Committee of Tzu Chi University.

Application of IH

The IH process was described in a previous study (23). In brief, rats were housed in Plexiglas cylindrical chambers (length 28 cm, diameter 10 cm, volume 2.4 l) with snug-fitting lids. Using a timed solenoid valve, pure nitrogen was distributed to the chambers for 30 s to reduce the ambient inspired O_2 fraction to 2%-6% for 2-5 s. This was followed by infusion of compressed air for approximately 45 s, allowing the gradual return of ambient air to enable an inspired O_2 fraction of 21%. Animals were exposed to IH between 1000 and 1600 h (6 h/day) for 12 consecutive days. This protocol was adopted because a previous study had shown that 12 days of IH exposure induced LV myocardial apoptosis in rats (7), and the number of days spent in the IH protocol matched that in the exercise protocol. This was also similar to a previous study that reported increased myocardial apoptosis in rats exposed to IH (4%-6% O_2 once per min, followed by 21% O_2 for 8 h/day) for 10 consecutive days (44).

Table 1. Body weight and core temperature of the rats

	n	Body Weight (g)		Core Temperature (°C)	
		Baseline	Day 12	Pre-Exercise	Post-Exercise
CON	10	332.1 ± 6.2	$373.4 \pm 6.2^{\dagger}$	37.3 ± 0.2	
IH	10	335.3 ± 5.5	$351.5 \pm 6.7^{*,\dagger}$	37.1 ± 0.2	
EXE	10	331.3 ± 6.2	$350.0 \pm 7.4^{*,\dagger}$	37.2 ± 0.3	$40.2 \pm 0.3^{\ddagger}$
IHEXE	10	329.5 ± 7.3	$349.5 \pm 10.5^{*,\dagger}$	37.1 ± 0.2	$40.0 \pm 0.2^{\ddagger}$

Values are presented as means ± SEM; n, number of rats; CON, controls; IH, intermittent hypoxia; EXE, exercise; IHEXE, IH interspersed with EXE. *$P < 0.05$ compared with CON group. $^{\dagger}P < 0.05$ compared with baseline. $^{\ddagger}P < 0.05$ compared with pre-exercise.

Exercise Training Protocol

The exercise protocol was described in a previous study (7). In brief, animals assigned to the exercise groups were habituated to treadmill exercise for 5 consecutive days, involving a gradual increase in running time, beginning with 10 min/day and ending with 50 min/day. After a 2-day rest, the animals performed 5 consecutive days of treadmill exercise for 60 min/day at 30 m/min on a 2% grade. Body temperatures were measured daily with an electronic rectal thermometer before and immediately after exercise to verify the efficacy of exercise for generating a hyperthermic response. This protocol provides increased body temperatures immediately after daily exercise training similar to our previous study, which reported that 5 consecutive days of exercise provided protection against IH-induced myocardial apoptosis in Sprague-Dawley rats (7).

Echocardiography for LV Function

Echocardiography was performed under anaesthesia (inhalation of 1.2%-1.5% isoflurane in oxygen), at baseline and after 12 days. A core temperature of 37.5°C was maintained during the measurement. Transthoracic M-mode images of the LV in parasternal short-axis views were obtained at the level of the papillary muscles using a high-resolution ultrasound probe. The directly measured parameters included heart rate, end-systolic dimension of the LV (LVDs) and end-diastolic dimension of the LV (LVDd). LV fractional shortening was calculated as [(LVDd − LVDs)/LVDd] × 100. LV ejection fraction (LVEF) was calculated by Teichholz's method as [(LV end-diastolic volume − LV end-systolic volume)/LV end-diastolic volume] × 100. LV end-diastolic volume was calculated as $\{[7/(2.4 + LVDd)] \times LVDd^3\}$, and LV end-systolic volume as $\{[7/(2.4 + LVDs)] \times LVDs^3\}$ (5).

Myocardial Tissue Preparation

All animals were sacrificed with intraperitoneal injections of 500 mg/kg urethane (Sigma, St. Louis, MO, USA). Following a midline skin incision, the chest plate was reflected to expose the heart and lungs. The heart was lifted slightly and removed by cutting the aortic arch beyond the left subclavian artery (1.0-1.5 cm from the heart). The heart was immediately arrested and immersed in an ice-cold phosphate-buffered saline (PBS) solution. After the heart stopped beating, it was removed from PBS, and the aorta was immediately affixed to a cannula attached to a syringe. The hearts were retrogradely perfused (pressure: 110 mm H_2O) with PBS for 1 min to wash out the blood. The LV myocardium was quickly removed, divided into 6 sections, snap frozen in liquid nitrogen and stored at -80°C until use. Care was taken to consistently harvest the same region of the LV myocardium from all animals.

Matrix Metalloproteinase-2 (MMP-2), MCP-1, TNF-α and IL-6 Assays

Myocardial inflammation was measured using commercially available TNF-α and IL-6 ELISA kits (KOMABIOTECH, Yeongdeungpo-gu, Seoul, Seoul, Korea) and MMP-2 and MCP-1 kits (Uscn Life Science, Wuhan, Hubei, PRC) according to the manufacturer's instructions. All measurements were performed in duplicates on the same microtiter plate using an ELISA reader (Perkin-Elmer, Waltham, MA, USA). Expression of MMP-2, TNF-α, IL-6 and MCP-1 was normalized to the total protein amount in the sample and reported as pg/mg protein in the LV myocardium sample.

Protein Extraction and Western Blot

The protocol was adapted and modified from our previous studies (6, 7). Total cytosolic fraction extracts were obtained from the LV myocardium using a commercially available isolation kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Total protein content was quantified in duplicates using a protein assay kit (Bio-Rad, Hercules,

CA, USA) and bovine serum albumin (Sigma) standards. Samples (20 μg) were resolved on 12% SDS-PAGE for 2 h at room temperature and then electrophoretically transferred onto PVDF membranes (Amersham, Piscataway, NJ, USA). The membranes were blocked with 5% non-fat milk in Tris-buffered saline with 0.05% Tween 20 (TBS-T) at room temperature for 1 h and probed with primary antibodies for mouse anti-NHE-1 (1:1,000, Chemicon, Billerica, MA, USA) and mouse anti-β-actin (1:10,000, Chemicon) diluted in TBS-T with 2% BSA. All primary antibody incubations were conducted at room temperature for 2 h. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h (1:1,000, anti-mouse IgG antibody, Chemicon), and the signals were developed by enhanced chemiluminescence (34080, Pierce, Rockford, IL, USA). The signals were visualized by exposing the membranes to X-ray films (Kodak, NY, USA). The resulting bands were captured by a scanner and quantified as arbitrary units (OD × band area) by ImageJ analysis software (National Institutes of Health, Bethesda, MD, USA). Protein expression in the LV myocardium was reported as the ratio of protein to β-actin.

Quantitative Real-Time Polymerase Chain Reaction (qPCR)

The protocol was adapted and modified from a previous study (18). In brief, total RNA was isolated using the RNeasy Mini Kit and RNase-Free Dnase Set (Qiagen, Valencia, CA, USA) and reverse transcribed using the Omniscript RT Kit (Qiagen) as described previously. The following primers were used: NHE-1, forward: 5′-ATGTGGCTGGGAAACA-AGAC-3′, reverse: 5′-GACAGTCCCTCCCGTG-TAAA-3′ (18); GAPDH forward: 5′-TGCACCAC-CAACTGCTTAGC-3′, reverse: 5′-GCCCCACG-GCCATCA-3′ (33). PCR was performed using the SYBR Green method using TaqDNA polymerase (Invitrogen, Carlsbad, CA, USA). Fluorescence data were acquired at the end of extension. The cycle threshold value was measured and calculated by computer software (ABI 7300 Real Time PCR system, Life Technologies, USA). We used 3 μg of total RNA to perform each reverse transcription. A 1:10 dilution of cDNA obtained in the reverse transcription reaction (25 μL total volume) was used in each qPCR. The comparative C_t method ($2^{-\Delta\Delta C_t}$) was used to quantify gene expression, where $\Delta\Delta C_t = \Delta C_t$ (sample) − ΔC_t (reference).

Statistical Analyses

Statistical analyses were performed using SPSS 13.0 software (SPSS, Inc. Chicago, IL, USA). All values are expressed as means and standard errors of means. One-way analysis of variance (ANOVA) was used to compare myocardial levels of MMP-2, MCP-1, TNF-α, IL-6 and NHE-1 mRNA and protein. Two-way ANOVA was used to compare experimental processes and whole body weight, body temperature, LVDd, LVDs, LVFS% and LVEF% of the rats. Tukey's HSD protected least-significant difference test was used to determine differences between the means when comparing >2 groups. Significance was established a priori at $P < 0.05$.

Results

Body weight was significantly increased in all groups on day 12 compared with day 0 (CON: 12.6%, IH: 4.8%, EXE: 5.7% and IHEXE: 6.1%) (Table 1) ($P < 0.05$). Average body weight in IH, EXE and IHEXE rats was significantly lower on day 12 by 5.9%, 6.3% and 6.4%, respectively, compared with CON rats ($P < 0.05$ in all groups). Average body temperature was increased significantly by 3.0°C in EXE ($P < 0.05$) and 3.1°C in IHEXE rats ($P < 0.05$) immediately after exercise.

Echocardiographic parameters were examined and comparing with the baseline, LVDd values on day 12 were not increased significantly in the CON, IH, EXE and IHEXE rats, respectively ($P > 0.05$ in all groups). LVDd measurements were not different between the CON, IH, EXE and IHEXE rats when any two groups were compared at the baseline and on day 12 ($P > 0.05$, Table 2). However, LVDs measurements were significantly increased by 23.2% on day 12 in IH rats compared with the baseline. On day 12, the average of LVDs parameters was 19.6% higher in the IH rats compared with the CON rats ($P < 0.05$). LVDs measurements were 19.2% and 24.0% lower in the EXE and IHEXE rats, respectively, compared with the IH rats ($P < 0.05$, Table 2). LVFS% did not differ between the CON, EXE and IHEXE rats on day 12 ($P > 0.05$) but was decreased significantly by 22.9% in the IH rats compared with the baseline. Average LVFS% did not differ between the EXE and IHEXE rats on day 12, ($P > 0.05$) but was 23.1% lower in the IH rats compared with the CON rats ($P < 0.05$). LVFS% measurements were 32.2% and 36.9% higher in the EXE and IHEXE rats, respectively, compared with the IH rats ($P < 0.05$, Table 2). LVEF% did not differ ($P > 0.05$) between the CON, EXE and IHEXE rats on day 12 and was decreased by 15.1% in the IH rats compared with the baseline ($P < 0.05$). The average of LVEF measurements did not differ between the EXE and IHEXE rats on day 12, ($P > 0.05$ in both groups) and was 15.2% lower in the IH rats compared with CON rats ($P < 0.05$). LVEF measurements were 18.1% and 20.1% higher in the EXE and IHEXE rats,

Table 2. Echocardiographic data

	CON (n = 10)	IH (n = 10)	EXE (n = 10)	IHEXE (n = 10)
LVDd, mm				
Baseline	7.42 ± 0.26	7.62 ± 0.28	7.68 ± 0.2	7.65 ± 0.22
Day 12	7.64 ± 0.33	7.28 ± 0.21	7.56 ± 0.27	7.36 ± 0.32
LVDs, mm				
Baseline	3.86 ± 0.23	3.53 ± 0.15	3.9 ± 0.12	3.58 ± 0.25
Day 12	3.62 ± 0.23	$4.33 \pm 0.17^{*,\dagger}$	$3.5 \pm 0.26^{\S}$	$3.29 \pm 0.31^{\S}$
LVFS, %				
Baseline	48.1 ± 2.1	53.3 ± 1.9	49.1 ± 1.4	53.2 ± 2.8
Day 12	52.9 ± 1.3	$40.7 \pm 1.1^{*,\dagger}$	$53.8 \pm 2.7^{\S}$	$55.7 \pm 3.2^{\S}$
LVEF, %				
Baseline	77.6 ± 1.9	82.5 ± 1.6	78.6 ± 1.4	81.8 ± 2.4
Day 12	82.3 ± 1.2	$69.8 \pm 1.3^{*,\dagger}$	$82.4 \pm 2.2^{\S}$	$83.8 \pm 2.2^{\S}$

Values are presented as means $\pm$ SEM. CON, controls; IH, intermittent hypoxia; EXE, exercise; IHEXE, IH interspersed with EXE. Echocardiographic parameters include the following: LVDd, end-diastolic dimension of the left ventricle; LVDs, end-systolic dimension of the left ventricle; LVFS, left ventricular fractional shortening; LVEF, left ventricular ejection fraction. *$P < 0.05$ compared with the CON group. $^{\dagger}P < 0.05$ compared with the baseline. $^{\S}P < 0.05$ compared with the IH group.
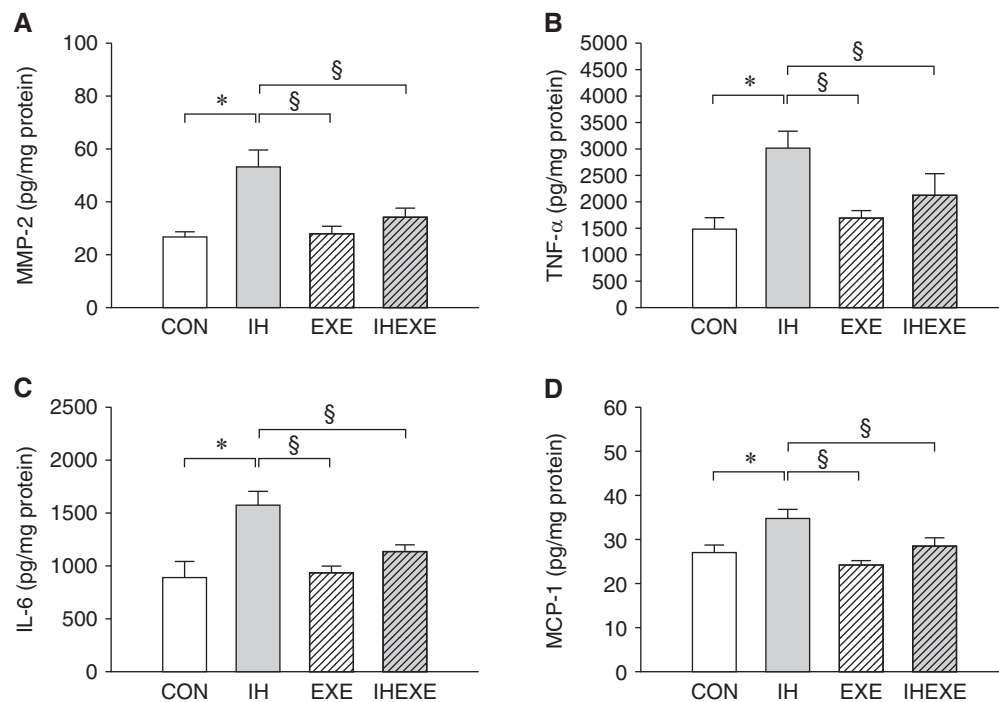

Fig. 1. Expression levels of matrix metalloproteinase-2 (MMP-2) (A), tumour necrosis factor-alpha (TNF-α) (B), interleukin-6 (IL-6) (C) and monocyte chemoattractant protein-1 (MCP-1) (D) in the left ventricles of control (CON), intermittent hypoxia (IH), exercise (EXE) and IH interspersed with EXE (IHEXE) rats. *$P < 0.05$ compared with CON group. $^{\S}P < 0.05$ compared with the IH group. Values are presented as means $\pm$ SEM (n = 10 per group).

respectively, compared with the IH rats ($P < 0.05$, Table 2).

Myocardial MMP-2 levels increased by 98.3% in the IH rats compared with CON rats ($P < 0.05$). The levels were lower in the EXE and IHEXE rats (47.4% and 35.6%, respectively) than in the IH rats ($P < 0.05$). However, the levels did not differ between the CON and EXE rats or between the CON and IHEXE
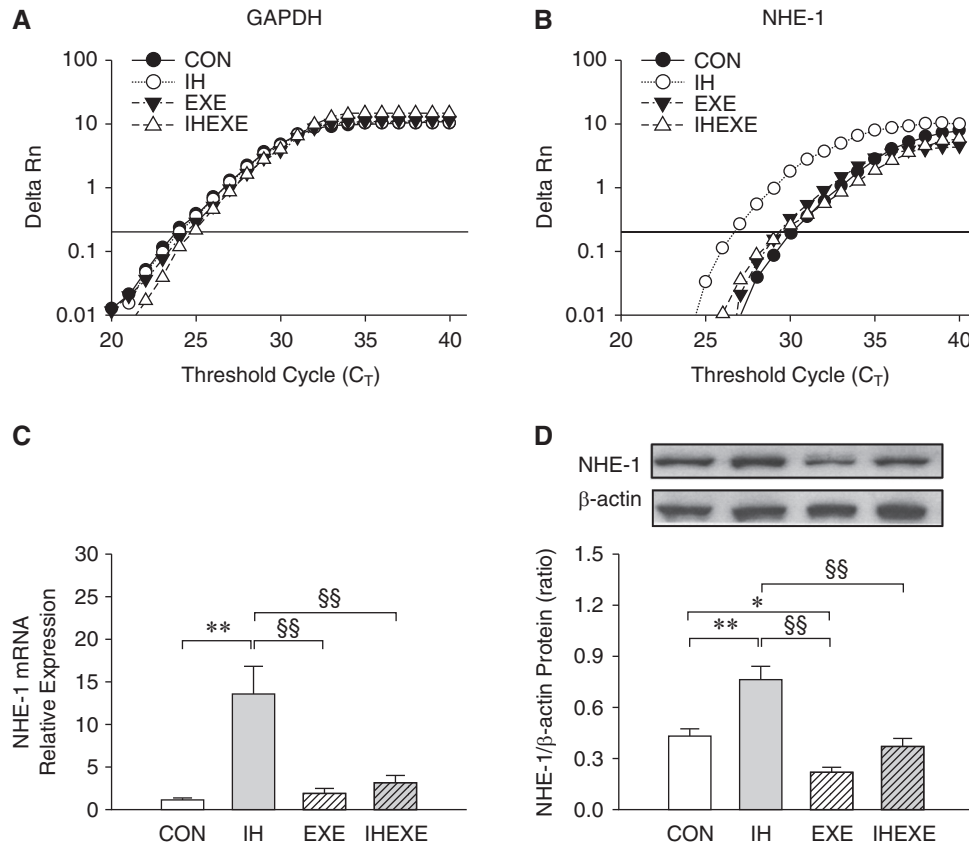
Fig. 2. Expression levels of sodium-hydrogen exchanger-1 (NHE-1) mRNA (A-C) and protein (D) in the left ventricles of control (CON), intermittent hypoxia (IH), exercise (EXE) and IH interspersed with EXE (IHEXE) rats. *$P < 0.05$; **$P < 0.01$ compared with CON group. §§$P < 0.01$ compared with the IH group. Values are presented as means ± SEM (n = 10 per group).

rats ($P > 0.05$; Fig. 1A). Myocardial TNF-α levels increased by 112.6% in the IH rats compared with the CON rats ($P < 0.05$) and decreased by 38.1% and 32.73% in the EXE and IHEXE rats, respectively, when compared with the IH rats ($P < 0.05$; Fig. 1B). Myocardial IL-6 levels increased by 64.9% in the IH rats compared with the CON rats ($P < 0.05$) and decreased by 33.5% and 21.8% in the EXE and IHEXE rats, respectively, when compared with the IH rats ($P < 0.05$; Fig. 1C). Myocardial MCP-1 levels increased by 28.7% in the IH rats compared with the CON rats ($P < 0.05$). MCP-1 levels decreased in the EXE and IHEXE rats by 30.0% and 17.8%, respectively, when compared with the IH rats ($P < 0.05$; Fig. 1D). Myocardial levels of MCP-1, TNF-α and IL-6 did not differ between the CON and EXE rats or between the CON and IHEXE rats ($P > 0.05$; Fig. 1, B-D).

Myocardial NHE-1 mRNA levels significantly increased by 10-fold in the IH rats compared with the CON rats ($P < 0.01$) and decreased by 85.6% in EXE and 76.4% in the IHEXE rats compared with the IH rats ($P < 0.01$). NHE-1 mRNA levels did not differ between the CON and EXE rats or between the CON and IHEXE rats ($P > 0.05$; Fig. 2, A-C). Myocardial

NHE-1 protein levels increased by 79.1% ($P < 0.01$) in the IH rats and decreased by 48.8% in the EXE rats compared with the CON rats ($P < 0.05$). The levels were 71.4% and 51.8% lower in the EXE and IHEXE rats, respectively, compared with the IH rats ($P < 0.01$). NHE-1 protein levels did not differ between the CON and IHEXE rats or between the EXE and IHEXE rats ($P > 0.05$; Fig. 2D).

Discussion

Our study showed that the LV function (LVFS% and LVEF%) deteriorated in IH with a parallel increase in TNF-α, IL-6 and MCP-1 levels. The deterioration was associated with up-regulation of NHE-1 mRNA expression and protein levels. Although exercise did not change pro-inflammatory factors and the expressed levels of myocardial NHE-1 mRNA compared with controls, short-term exercise could reduce myocardial NHE-1 protein levels. We showed that in IHEXE rats, short-term exercise reduced the negative effect of IH on the myocardium, including a reduction in LV dysfunction, improvement in systolic function, decrease in the levels of myocardial inflammatory

markers such as TNF-α, IL-6 and MCP-1 and attenuation of the expression of NHE-1 mRNA and protein in the myocardium.

During obstructive apnoeas, negative inspiratory intrathoracic pressure generated against the occluded pharynx increases LV transmural pressure, leading to increased afterload. In addition, increased LV transmural pressure increases myocardial oxygen demand, concurrently reducing coronary blood flow during which apnoea-related hypoxia reduces oxygen delivery. This can precipitate myocardial ischemia and impair cardiac contractility and diastolic relaxation (11, 20, 35). In animal studies, both 10 consecutive days (44) and months (4, 5) of IH exposure were shown to exhibit similar deleterious effects on myocardial contractile function, including higher LVDs and lower LVFS% and LVEF%. In adult male Wistar rats exposed to 25 days of chronic intermittent high altitude (IHA) hypoxia in a hypobaric chamber, significantly increased MMP-2 activation was observed in the right ventricle but not in the LV (37). The IH profiles in our study were different from those of IHA, and our findings showed that myocardial MMP-2 levels were increased by IH. Increased MMP-2 activation can contribute to mechanical dysfunction of the heart (8).

Exercise training increases LVEF%, cardiac output and ventilatory pattern, and ameliorates peripheral chemoreflex control in HF patients (40). As addition samples, 3-10 weeks of exercise training could protect the heart under pathological conditions such as ischemia/reperfusion injury (30, 32), post-myocardial infraction (13), hypertension (45) and type II diabetes mellitus (34), despite that these effects were not necessarily manifesting under physiological conditions. In this study, the lower LV function, determined by higher LVDs and lower LVFS% and LVEF%, and the higher MMP-2 levels in the IH-exposed rats suggested that 12 days of IH exposure can yield deleterious effects on LV contractile function. However, improved LV function associated with reduced MMP-2 levels were found in IHEXE rats, which may indicate that short-term exercise can provide beneficial effects against IH-induced LV dysfunction by attenuating the expression of myocardial MMP-2.

On the other hand, OSA has been repeatedly shown to be associated with elevated markers of inflammation (42, 43), and a transient increases in TNF-α and IL-6 levels induced by IH have implications for the pathogenesis of LV dysfunction in OSA (38). Enhanced myocardial TNF-α and IL-6 levels by short-term IH demonstrated in our study is similar to the findings of a previous study demonstrating that activation of pro-inflammatory cytokine pathways could cause functional alteration of the carotid body under only 7 days of IH treatment (24). In addition, it was demonstrated that exercise and diet intervention in

C57BL/6 mice could attenuate increased myocardial MCP-1 levels caused by high-fat diet (19). Moreover, a previous study reported that exercise training inhibited the isoprenaline-induced increase in myocardial levels of TNF-α and IL-6 mRNA. However, exercise training did not affect local cytokines in the untreated group (33). In our study, exercise did not alter myocardial levels of MCP-1, TNF-α and IL-6 in controls, but there were significantly lower levels of MCP-1, TNF-α and IL-6 in the IHEXE rats compared with the IH rats. Our results demonstrated that short-term exercise could prevent IH-induced myocardial inflammation without changes in the physiological condition.

A previous study reported that aerobic exercise training increased NHE-1 activity and decreased myocardial NHE-1 levels at physiological intracellular pH and attenuated H_2O_2-mediated activation of NHE1 activity (16). In this study, exercise attenuated myocardial levels of NHE-1 protein, and there were lower myocardial levels of NHE-1 protein and mRNA in the IHEXE rats compared with the IH rats. Our result demonstrated that short-term exercise could prevent IH-induced NHE-1 activation. Taken together, inhibition of NHE-1 activation can reduce the expression of pro-inflammatory cytokines (26). Increased levels of TNF-α, IL-6 and MCP-1 can worsen LV function (21, 27). Our results of lower myocardial levels of NHE-1 protein and mRNA accompanied by lower myocardial levels of MCP-1, TNF-α and IL-6 in the IHEXE rats compared to the IH rats suggested that exercise attenuated myocardial levels of NHE-1 and reduced the IH-induced cardiac inflammation. These results might contribute to that IH-induced LV dysfunction prevented by exercise.

In this study, IH pattern (2%-6% O_2 for 2-5 s per 75 s, followed by 21% O_2) is commonly seen in OSA and is clinically the most relevant pattern (6, 23, 31). The exercise model was selected according to a training program that promotes myocardial adaptation in adult male rats (15, 30). Heart response to cardioprotection through exercise can be quite different depending upon exercise duration and intensity (14). Short-term *vs*. long-term training may provide different cardioprotective effects. However, emerging evidenced indicate that NHE-1 may be activated by reactive oxygen species (ROS). This pathway of activation involves extracellular signal-regulated kinases 1 and 2 (ERK1/2)- and p90rsk-dependent phosphorylation (41). Increased ROS production may be induced by the rise in $[Na^+]_i$ secondary to NHE-1 hyperactivity (12). Thus, future studies should direct attention to the regulation mechanisms of NHE-1 to investigate that exercise provides beneficial effects to prevent IH-induced NHE-1 hyperactivity.

In conclusion, our results revealed that 5 consecutive days of exercise provided potent cardiopro-

tective effects against IH-induced LV dysfunction. Exercise may exert the protective effect by inhibiting activation of myocardial NHE-1 protein that, in part, plays a role to attenuate the secretion of myocardial pro-inflammatory cytokines induced by IH.

Acknowledgments

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