Chronic intermittent hypobaric hypoxia decreases β-adrenoceptor activity in right ventricular papillary muscle

Yue Guan,1 Lu Gao,1 Hui-Jie Ma,1 Qian Li,1 Hao Zhang,1 Fang Yuan,1 Zhao-Nian Zhou,2 and Yi Zhang1
1Department of Physiology, Hebei Medical University, Shijiazhuang, China; and 2Laboratory of Hypoxic Cardiovascular Physiology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

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Guan Y, Gao L, Ma H, Li Q, Zhang H, Yuan F, Zhou Z, Zhang Y. Chronic intermittent hypobaric hypoxia decreases β-adrenoceptor activity in right ventricular papillary muscle. Am J Physiol Heart Circ Physiol 298: H1267–H1272, 2010. First published January 22, 2010; doi:10.1152/ajpheart.00410.2009.—Chronic intermittent hypobaric hypoxia (CIHH) has an effective cardiac protection against ischemia-reperfusion injury. However, the underlying mechanisms are not fully known. It has been shown that blockade of β-adrenergic receptor exerts anti-arrhythmic action and improves cardiac remodeling in ischemic myocardium. Thus we determined the influence of CIHH on β-adrenergic receptor activity in right ventricular papillary muscle of rats. We found that the action potential duration in right ventricular papillary muscle was significantly longer in CIHH rats than in control rats. Activation of β-adrenergic receptor with dl-isoproterenol dose-dependently increased action potential duration and the contractility in right ventricular papillary muscle. In CIHH rats, the prolonged effect of dl-isoproterenol on action potential duration and the positive inotropic effect were significantly decreased compared with that in control rats. Furthermore, radioligand-binding experiments revealed that the density and affinity of β-adrenergic receptor in right ventricular myocardium was significantly lower in CIHH rats. In addition, Western blot analysis revealed that the membrane-bound G protein Go expression level in cardiac myocardium was significantly lower in CIHH rats than that in control rats. Collectively, these data suggest that CIHH suppresses β-adrenergic receptor action in right ventricular papillary muscle through decreasing receptor density and affinity, as well as membrane-bound Go. This mechanism may be involved in the cardiac protective effect of CIHH.

MATERIALS AND METHODS

Animals and CIHH treatment. All experiments were carried out in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and was reviewed and approved by the Ethics Committee for the Use of Experimental Animals at Hebei Medical University. Twenty-four age- and body weight-matched male Sprague-Dawley rats (provided by the Experimental Animal Center of Hebei Province, China) were divided into the following four groups: control group (Con, n = 6), CIHH treatment for 14 days (CIHH14, n = 6), CIHH treatment for 28 days (CIHH28, n = 6), and CIHH treatment for 42 days (CIHH42, n = 6). Rats in CIHH groups were exposed to intermittent hypoxia in a hypobaric chamber at 5,000 m altitude (Pa = 404 mmHg, Po2 = 84 mmHg) for 6 h daily (from 10:00 A.M. to 4:00 P.M.) for 14, 28, and 42 days, respectively. Control rats were under normoxic environmental conditions. Standard rodent diet and tap water were available ad libitum to all rats. Body weights of rats were measured weekly.

Preparation of cardiac right ventricular papillary muscle. The right ventricular papillary muscle was prepared as described previously (23). Briefly, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The hearts were quickly removed and rinsed in ice-cold modified Tyrode’s solution saturated by 100% O2. The Tyrode’s solution contained (in mmol/l): 136.8 NaCl, 5.4 KCl, 1.05 MgCl2, 1.2 NaHCO3, 11.0 glucose, and 5.0 Tris (pH 7.4 ± 0.05, gassed with 100% O2). One end of the papillary muscle (isolated from the right ventricles) was fixed on the bottom of a small chamber by a micropin. The other end was connected to a force transducer (JY100; XIHANG) for measuring the muscle tension. The papillary muscle was continuously perfused with modified Tyrode’s solution at 12 ml/min at 37°C for at least 1 h before the experiments.

Action potential and contraction recording. The action potential was elicited by electrical stimulation at a frequency of 1 Hz and intensity of twofold threshold that induced action potential. The action potential was measured and recorded using the multi-channel bioelectric recorder B1200 (BIO-LOG). The action potential duration was defined as the time required for the action potential to decline to 90% of the peak amplitude from the threshold level. The contraction was measured by a transducer (0.01 g force units, JZ100) attached to the free end of the isometric muscle.

RESULTS

Long-TERM HIGH-ALTITUDE HYPOXIA can protect the heart against ischemia/hypoxia injury, including anti-arrhythmia and reduction of infarct size during acute ischemia (10). Chronic intermittent hypobaric hypoxia (CIHH), similar to the concept of ischemic preconditioning, also has significant protective effect on the heart against ischemia-reperfusion injury (1, 21, 28). Previous studies have demonstrated that CIHH inhibits ischemia- and reperfusion-induced Ca2+ overloading in cardiac myocyte, preserves contractility of myocardium, and prevents apoptosis of cardiomyocytes (7, 22, 24). Multiple mechanisms or pathways are involved in the cardioprotection of CIHH. These mechanisms include the induction of heat-shock protein 70 (5, 25), increases in coronary flow and myocardial capillary angiogenesis (26), activation of ATP-sensitive K+ channels, inhibition of mitochondrial permeability transition pores (27, 28), and activation of protein kinase C (6). However, the mechanisms underlying the anti-arrhythmic effect of CIHH are not fully known.

The sympathetic nervous system is critically involved in the regulation of cardiac function through β-adrenergic receptors in both physiological and pathological situations, for instance, sympathetic hyperactivity during acute myocardial ischemia may lead to malignant arrhythmias and infarction (4). Zicha et al. (29) reported that β-adrenergic receptor blockade could improve cardiac remodeling and have an anti-arrhythmic effect in ischemic myocardium. Also, the activity of β-adrenergic receptor can be changed during hypoxia (12, 13, 15, 16, 19). Little is known, however, whether β-adrenergic receptors play a role in CIHH cardiac protection.

The aim of present study was to explore the effect of CIHH on the activity of β-adrenergic receptor in myocardium and underlying the mechanism. We hypothesized that decreased β-adrenergic receptor function contributes to the cardiac protection produced by CIHH treatment.

action potential; contraction; intermittent hypoxia; β-adrenergic receptor; G protein

Address for reprint requests and other correspondence: Y. Zhang, Dept. of Physiology, Hebei Medical Univ., 361 Zhong Shan Dong Rd., Shijiazhuang Hebei 050017, China (e-mail: zhyhenry@hotmail.com).
stimuli were delivered through a bipolar electrode placed in the chamber and connected to a stimulator (YC-2; Chengdu Instrument Factory). Action potentials were recorded with glass microelectrodes filled with 3 M KCl and fed into a high-impedance microelectrode amplifier (SFW-1; Chengdu Instrument Factory). Resting potential (RP), action potential overshoot (OS), amplitude (APA), maximal rising rate of phase 0 (V_{\text{max}}), and width at 50% and 90% repolarization (APD_{50} and APD_{90}, respectively) were measured and stored in a computer hard drive. The papillary muscle contractility was induced by the stimulation. The muscle contraction was recorded, and parameters of contraction including maximal isometric tension (\(P_{\text{max}}\)) and velocity of tension development (\(P_{\text{dT/dt}}\)) were analyzed with a self-designed program.

Radioactive ligand binding assay. Rough membranes from the right ventricle of heart were homogenized in 10 ml PBS (20 mM sodium phosphate, 154 mM NaCl; pH 7.6) at 4°C. After being filtered by a four-layer absorbent gauze, the suspension was centrifuged at 20,000 \(g\) for 10 min, and the pellet was resuspended in another 10 ml PBS and centrifuged at 20,000 \(g\) for a further 10 min at 4°C. The pellet was finally resuspended in 5 ml PBS and kept on ice until use. Protein concentration was determined by the BCA methods using bovine serum albumin as standard. The \(G_{o}\) subunit protein expression in right ventricle in Con and CIHH rats. Membrane proteins were prepared by the Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit (Pierce) according to the manufacturer’s protocols. For immunoblotting of \(G_{o}\) proteins, SDS gel electrophoresis of polypeptides was performed on 8% polyacrylamide gels. Samples, each containing 30 \(\mu\)g protein, were added with the same amount of the sample-loading buffer (the reducing buffer), which was heated for 5 min at 100°C. The solution was added into a single lane of the gel. After gel running, electrophoresis was performed, during which polypeptides were transferred to a nitrocellulose membrane. The membrane was then washed with a Tris-buffered saline solution (TBS) and incubated with TBS that contained 5% nonfat dry milk (blocking buffer), a procedure that blocks the nonspecific protein binding sites on nitrocellulose. The \(G_{o}\) protein antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:500 dilution in the blocking buffer was incubated with the membrane for 4 h at room temperature. After three wash with TBS containing 0.5% Tween 20, the nitrocellulose was incubated with alkaline phosphatase-conjugated goat antirabbit IgG diluted at 1:5,000 in the blocking buffer at 4°C. The protein level on the nitrocellulose membrane was detected by a chemiluminescent substrate system. The nitrocellulose membrane was sealed with a working solution, which was prepared by mixing equal volumes of reagents A and B in the assay kit (Fuji) for further developing and fixing to the X-ray film (Kodak, Rochester, NY). Table 1. Effects of CIHH on action potential and contraction in right ventricular papillary muscle of rats

<table>
<thead>
<tr>
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<th>Action Potential</th>
<th>Contraction</th>
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<tr>
<td></td>
<td>(\text{RP}, \text{mV})</td>
<td>(\text{OS, mV})</td>
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<tr>
<td>Con</td>
<td>(-80.7 \pm 1.1)</td>
<td>(11.0 \pm 0.8)</td>
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<tr>
<td>CIHH14</td>
<td>(-80.6 \pm 1.4)</td>
<td>(11.3 \pm 1.5)</td>
</tr>
<tr>
<td>CIHH28</td>
<td>(-80.6 \pm 1.3)</td>
<td>(11.2 \pm 2.1)</td>
</tr>
<tr>
<td>CIHH42</td>
<td>(-80.0 \pm 0.8)</td>
<td>(10.3 \pm 1.4)</td>
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Values are means ± SD; \(n = 6\) animals in each group. RP, resting potential; OS, overshooting; APA, amplitude of action potential (AP); \(V_{\text{max}}\), maximal rate of 0 phase depolarization; APD\(_{50}\), AP 50% repolarization; APD\(_{90}\), AP 90% repolarization; \(P_{\text{max}}\), maximal isometric tension; \(P_{\text{dT/dt}}\), velocity of tension development. *\(P < 0.05\) and †\(P < 0.001\) vs. control values.
Densitometric analysis was conducted on the protein bands for quantitative comparison. All data were expressed as means ± SD. Comparisons among multigroups were evaluated with a one-way ANOVA followed by Dunnett’s test when several groups were compared with a single control group. A value of $P < 0.05$ was considered significant.

**RESULTS**

**Body weight and heart weight.** The body weight of rats in CIHH groups had no significant change compared with those in the control group. The ratios of ventricle (including whole, left, and right) weight to body weight were not significantly different between Con and CIHH groups (Fig. 1.).

**Action potential duration and contractility in right papillary muscle in CIHH rats.** We first determined the APD$_{50}$ in right ventricular papillary muscle in both Con and CIHH rats. The value of APD$_{50}$ was $31.5 \pm 6.5$, $34.8 \pm 7.4$ ($P < 0.01$), and $47.3 \pm 8.1$ ms ($P < 0.01$) in CIHH14, CIHH28, and CIHH42 rats, respectively, significantly longer than $23.2 \pm 5.5$ ms in Con rats. The value of APD$_{50}$ was $99.5 \pm 5.0$, $100.8 \pm 9.0$, and $132.7 \pm 20.7$ ms in CIHH14, CIHH28, and CIHH42 rats, respectively, significantly longer than $68.8 \pm 7.1$ ms in Con rats ($P < 0.05$). RP, OS, APA, and $V_{\text{max}}$ did not differ significantly between Con and CIHH groups. In addition, there was no difference in contractility of right papillary muscle from CIHH and Con rats (Table 1 and Fig. 2).

**Effects of DL-isoproterenol on action potential and contractility in right papillary muscle in CIHH rats.** We then determined the response of action potential and contractility in right papillary muscle to activation of $\beta$-adrenergic receptors. In this set of experiments, different concentrations ($10^{-8}$, $10^{-7}$, and $10^{-6}$ mol/l) of DL-isoproterenol were cumulatively applied to the recording chamber. Action potential and contraction in right papillary muscle were recorded 3 min before and after drug application. DL-Isoproterenol increased the APA, OS, and $V_{\text{max}}$ in a dose-dependent manner. Furthermore, APD$_{50}$ and APD$_{90}$ in right papillary muscle were significantly prolonged.

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![Fig. 2. Original recording of action potential on DL-isoproterenol (ISO) in right ventricular papillary muscle of rats. a, Baseline; b, $10^{-8}$ mol/l ISO; c, $10^{-7}$ mol/l ISO; d, $10^{-6}$ mol/l ISO.](image)

**OS (%)**

![Fig. 3. Effects of ISO on action potential in right ventricular papillary muscle of rats. OS, overshooting; APA, amplitude of action potential; $V_{\text{max}}$, maximal rate of 0 phase depolarization; APD$_{50}$, 50% action potential repolarization; APD$_{90}$, 90% action potential repolarization. *$P < 0.05$ vs. baseline. $^*P < 0.05$ and $^{**}P < 0.01$ vs. control. Means ± SD, $n = 6$ in each group.](image)
Density and affinity of myocardial β-adrenergic receptor in CIHH and Con rats. We then determined the density and affinity of myocardial β-adrenergic receptor by using radioligand binding. We found that the density, expressed as Bmax, of β-adrenergic receptor in right ventricular myocardium was 45.8 ± 11.9 (n = 4), 25.1 ± 7.4 (P < 0.01, n = 4), and 29.1 ± 9.9 (P < 0.01, n = 4) fmol/mg in CIHH14, CIHH28, and CIHH42 rats, respectively. These values were significantly lower than 56.7 ± 150.4 (P < 0.05, n = 6) in Con rats. Meanwhile, we found the affinity (Kd) of β-adrenergic receptor in right ventricular myocardium was 344.4 ± 150.4 (P < 0.05, n = 4) and 427.6 ± 148.9 (P > 0.05, n = 4) pM in CIHH28 and CIHH42 rats, respectively. These values were lower than 767.2 ± 234.5 pM (n = 6) in Con rats. However, adaptation to CIHH, the affinity of β-adrenergic receptor in right ventricular myocardium in CIHH14 (801.5 ± 271.9 pM, n = 4), raised slightly (P > 0.05) (Fig. 5).

Expression level of myocardial Gsα protein isoforms in CIHH and Con rats. Because β-adrenergic receptors are coupled to Gsα protein α-subunits (45 kDa), we determined the Gsα expression level in the membrane of cardiomyocytes in CIHH and Con rats by using Western blot analysis. We found that the expression level and affinity of myocardial Gsα protein isoforms in CIHH rats were lower than in Con rats (P < 0.05, n = 6; Fig. 4).

DISCUSSION

This is the first study to find that the function of β-adrenergic receptor was decreased in CIHH-treated rats. We found that action potential duration was longer in right ventricular papillary muscle in CIHH rats. However, the basal contractility of ventricular papillary muscle was not different between CIHH and Con rats. Furthermore, we found that the dl-isoproterenol-induced prolongation of action potentials in right ventricular papillary muscle was attenuated in CIHH rats. Also, the positive inotropic effect of dl-isoproterenol in right ventricular papillary muscle was significantly attenuated in CIHH rats. In addition, we found the density and affinity of β-adrenergic receptor and expression level of Gsα protein α-subunits were decreased in right ventricular myocardium. These data suggested that CIHH treatment decreased activity of β-adrenergic receptors in right ventricular myocardium.

The sympathetic nervous system is critically involved in the regulation of cardiac function through β-adrenergic receptors. Activation of β-adrenergic receptors (β1-receptors) results in augmentation of cardiac activity (positive inotropic effect), including an increase in heart rate and atria-ventricle conduction velocity and enhancement of myocardial contraction. The sympathetic hyperactivity during acute myocardial ischemia,
however, may lead to arrhythmias and an increase in infarction size (4). Thus increased sympathetic activation has been recognized as a predictor of poor prognosis in heart failure patients (4, 20). In this regard, β-adrenergic receptor blockade improves cardiac remodeling and has an antiarrhythmic effect in ischemic myocardium (29). We found that the activity of β-adrenergic receptor in right ventricular myocardium was decreased in CIHH rats. This attenuation of β-adrenergic receptor activity may contribute to CIHH cardioprotection, at least in right ventricle, against ischemia- and reperfusion-induced cardiac injuries such as arrhythmia and infarction.

Previous studies have shown that hypoxia alters expression of β-adrenergic receptor in myocardium. However, the effects of hypoxia on expression of β-adrenergic receptor are inconsistent, for example, chronic hypoxia decreased the density of β-adrenergic receptor in heart (12, 13, 15, 16, 19). However, others found chronic hypoxia increased (14) or intermittent hypoxia had no effect (8, 9) on β-adrenergic receptor in myocardium. In our experimental condition, the density and affinity of β-adrenergic receptor were reduced in right ventricular myocardium of CIHH rats compared with Con rats, which suggested that the decrease of β-adrenergic receptor activity was the result of the reduction of density and affinity of β-adrenergic receptor. It also suggested that the effect of intermittent hypoxia on β-adrenergic receptor depended on the different model and level of hypoxia (2).

Cardiac hypertrophy was another factor affecting β-adrenergic receptor in myocardium. It has been shown that cardiac hypertrophy was often accompanied by the alteration of G protein-adenylate cyclase signaling (17). Böhm (3) reported that Gα expression increased and the number of β-adrenergic receptors was reduced in hypertrophic cardiomyopathy. In our experiment, we did not find hypertrophy in CIHH rats, which confirmed that the alteration in β-adrenergic receptor in right ventricular myocardium resulted from an effect of CIHH, not hypertrophy.

β-Adrenergic receptor is coupled to a G protein signaling pathway. Activation of β-adrenergic receptor increases cytoplasm cycle adenosine monophosphate and open Ca2+ channels. The decrease in the stimulatory G protein proteins may result in depression of β-adrenergic receptor function via diminishing signal transduction of β-adrenergic receptor. It was reported that the reduced β-adrenergic receptor activity was due partly to an impaired function of the Gs protein in chronic hypoxia heart (18). Similarly, Kacimi and coworkers (11) reported that...

Fig. 5. Density and affinity of β-adrenoceptor in right ventricular myocardium. A: β-adrenoceptor density and affinity of Con (n = 6), CIHH14 (n = 4), CIHH28 (n = 4), and CIHH42 (n = 4). B: relative changes showing β-adrenoceptor density and affinity in right ventricular myocardium of Con and CIHH rats. Kd, dissociation constant; Bmax, maximal bound capacity. *P < 0.05 and **P < 0.01 vs. control. Means ± SD.

Fig. 6. Immunoblot analysis of Gsα in myocardial membranes of CIHH rats. A: representative Western blot of Gsα and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in right ventricles of Con and CIHH groups. B: the relative changes in Gsα levels were assessed by densitometric scanning. The levels were normalized to GAPDH. *P < 0.05 vs. control. Means ± SD, n = 4 rats in each group.
functional activity of myocardial Gs protein was attenuated in chronic hypoxia-treated animals. On the other hand, there was a report that chronic hypoxia did not appreciably affect the content of the stimulatory G protein (9). In the present study, we found that the biologically active isoform, Gs protein (45 kDa), was reduced in myocardium in CIHH rats, suggesting that the reduced activity of β-adrenergic receptor was related with the reduction of Gs protein.

In summary, the present study has provided evidence for the first time that CIHH attenuates β-adrenergic receptor activity by decreasing β-adrenergic receptor density, affinity, and Gs protein in right ventricle of rats. These alterations of β-adrenergic receptor may contribute to cardiac protection in CIHH rats.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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