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

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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-adrenoceptor exerts anti-arrhythmic action and improves cardiac remodeling in ischemic myocardium. Thus we determined the influence of CIHH on β-adrenoceptor 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-adrenoceptor 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-adrenoceptor in right ventricular myocardium was significantly lower in CIHH rats. In addition, Western blot analysis revealed that the membrane-bound G protein Gα1 expression level in cardiac myocardium was significantly lower in CIHH rats. 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, Poe2 = 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·HCl (pH 7.4 ± 0.05, gassed with 100% O2). One end of the papillary muscle (isolated from the right ventricle) was fixed on the bottom of a small chamber by a micropip. The other end was connected to a force transducer (JZ100; XINHANG) 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
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 (SWF-1; Chengdu Instrument Factory). Resting potential (RP), action potential overshoot (OS), amplitude (APA), maximal rising rate of phase 0 (Vmax), and at width at 50% and 90% repolarization (APD50 and APD90, 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 (Pmax) and velocity of tension development (Pdtd) 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 protein concentration in each group.

Values are means ± SD; n = 6 animals in each group.

Table 1. Effects of CIHH on action potential and contraction in right ventricular papillary muscle of rats

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<thead>
<tr>
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<th>Action Potential</th>
<th>Contraction</th>
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<tr>
<td></td>
<td>RP, mV</td>
<td>OS, mV</td>
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<tr>
<td>Con</td>
<td>-80.7±1.1</td>
<td>11.0±0.8</td>
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<tr>
<td>CIHH14</td>
<td>-80.6±1.4</td>
<td>11.3±1.5</td>
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<tr>
<td>CIHH28</td>
<td>-80.6±1.3</td>
<td>11.2±2.1</td>
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<tr>
<td>CIHH42</td>
<td>-80.0±0.8</td>
<td>10.3±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); Vmax, maximal rate of 0 phase depolarization; APD50, AP 50% repolarization; APD90, AP 90% repolarization; Pmax, maximal isometric tension; Pdtd, velocity of tension development. *P < 0.05 and †P < 0.001 vs. control values.

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Densitometric analysis was conducted on the protein bands for quantitative comparison. Statistics. 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 ± 6.5, 34.8 ± 7.4 ($P < 0.01$), and 47.3 ± 8.1 ms ($P < 0.01$) in CIHH14, CIHH28, and CIHH42 rats, respectively, significantly longer than 23.2 ± 5.5 ms in Con rats. The value of APD$_{90}$ was 99.5 ± 5.0, 100.8 ± 9.0, and 132.7 ± 20.7 ms in CIHH14, CIHH28, and CIHH42 rats, respectively, significantly longer than 68.8 ± 7.1 ms in Con rats ($P < 0.05$). RP, OS, APA, and $V_{max}$ 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 β-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 $V_{max}$ in a dose-dependent manner. Furthermore, APD$_{50}$ and APD$_{90}$ in right papillary muscle were significantly prolonged.

**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.

**Fig. 3.** Effects of ISO on action potential in right ventricular papillary muscle of rats. OS, overshooting; APA, amplitude of action potential; $V_{max}$, 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.
potential were significantly less in CIHH rats than in Con rats (Figs. 2 and 3). In addition, DL-isoproterenol produced a dose-dependent increase in contraction in right papillary muscle. The $P_{\text{max}}$ and $P_{\text{dT/dt}}$ of contraction were significantly increased. Similarly, the DL-isoproterenol-induced increase in the contraction was significantly smaller in CIHH rats than in Con rats ($P < 0.05$, $n = 6$; Fig. 4).

**Density and affinity of myocardial $\beta$-adrenergic receptor in CIHH and Con rats.** We then determined the density and affinity of myocardial $\beta$-adrenergic receptor by using radioligand binding. We found that the density, expressed as $B_{\text{max}}$, of $\beta$-adrenergic receptor in right ventricular myocardium was $45.8 \pm 11.9$ (mean $\pm$ SD, $n = 4$), $25.1 \pm 7.4$ ($P < 0.01$, $n = 4$), and $29.1 \pm 9.9$ ($P < 0.01$, $n = 4$) fmol/mg in CIHH14, CIHH28, and CIHH42 rats, respectively. These values were significantly lower than $56.7 \pm 150.4$ fmol/mg in CIHH14, CIHH28, and CIHH42 rats, respectively. These values were significantly lower than $56.7 \pm 150.4$ fmol/mg in CIHH14, CIHH28, and CIHH42 rats, respectively.

**Expression level of myocardial $G_{\alpha}$ protein isoforms in CIHH and Con rats.** Because $\beta$-adrenergic receptors are coupled to $G_{\alpha}$ protein $\alpha$-subunits (45 kDa), we determined the $G_{\alpha}$ expression level in the membrane of cardiomyocytes in CIHH and Con rats by using Western blot analysis. We found that the expression level of $G_{\alpha}$ protein in right ventricular myocardium in CIHH14 (801.5 ± 271.9 pmol, $n = 4$), raised slightly ($P > 0.05$) (Fig. 5).

**DISCUSSION**

This is the first study to find that the function of $\beta$-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 $\beta$-adrenergic receptor and expression level of $G_{\alpha}$ protein $\alpha$-subunits were decreased in right ventricular myocardium. These data suggested that CIHH treatment decreased activity of $\beta$-adrenergic receptors in right ventricular myocardium.

The sympathetic nervous system is critically involved in the regulation of cardiac function through $\beta$-adrenergic receptors. Activation of $\beta$-adrenergic receptors ($\beta_1$-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, $\beta$-adrenergic receptor blockade improves cardiac remodeling and has an antiarrhythmic effect in ischemic myocardium (29). We found that the activity of $\beta$-adrenergic receptor in right ventricular myocardium was decreased in CIHH rats. This attenuation of $\beta$-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 $\beta$-adrenergic receptor in myocardium. However, the effects of hypoxia on expression of $\beta$-adrenergic receptor are inconsistent, for example, chronic hypoxia decreased the density of $\beta$-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 $\beta$-adrenergic receptor in myocardium. In our experimental condition, the density and affinity of $\beta$-adrenergic receptor were reduced in right ventricular myocardium of CIHH rats compared with Con rats, which suggested that the decrease of $\beta$-adrenergic receptor activity was the result of the reduction of density and affinity of $\beta$-adrenergic receptor. It also suggested that the effect of intermittent hypoxia on $\beta$-adrenergic receptor depended on the different model and level of hypoxia (2).

Cardiac hypertrophy was another factor affecting $\beta$-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 $G_\alpha$ expression increased and the number of $\beta$-adrenergic receptors was reduced in hypertrophic cardiomyopathy. In our experiment, we did not find hypertrophy in CIHH rats, which confirmed that the alteration in $\beta$-adrenergic receptor in right ventricular myocardium resulted from an effect of CIHH, not hypertrophy.

$\beta$-Adrenergic receptor is coupled to a $G_\alpha$ protein signaling pathway. Activation of $\beta$-adrenergic receptor increases cytoplasm cycle adenosine monophosphate and open Ca$^{2+}$ channels. The decrease in the stimulatory $G_\alpha$ proteins may result in depression of $\beta$-adrenergic receptor function via diminishing signal transduction of $\beta$-adrenergic receptor. It was reported that the reduced $\beta$-adrenergic receptor activity was due partly to an impaired function of the $G_\alpha$ protein in chronic hypoxia heart (18). Similarly, Kacimi and coworkers (11) reported that

![Fig. 5. Density and affinity of $\beta$-adrenoceptor in right ventricular myocardium. A: $\beta$-adrenoceptor density and affinity of Con ($n = 6$), CIHH14 ($n = 4$), CIHH28 ($n = 4$), and CIHH42 ($n = 4$). B: relative changes showing $\beta$-adrenoceptor density and affinity in right ventricular myocardium of Con and CIHH rats. $K_d$, dissociation constant; $B_{max}$, maximal bound capacity. *$P < 0.05$ and **$P < 0.01$ vs. control. Means ± SD.](http://ajpheart.physiology.org/)

![Fig. 6. Immunoblot analysis of $G_\alpha$ in myocardial membranes of CIHH rats. A: representative Western blot of $G_\alpha$ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in right ventricles of Con and CIHH groups. B: the relative changes in $G_\alpha$ 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.](http://ajpheart.physiology.org/)
functional activity of myocardial $G_\alpha$ 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, $G_\alpha$ (45 kDa), was reduced in myocardium in CIHH rats, suggesting that the reduced activity of $\beta$-adrenergic receptor was related with the reduction of $G_\alpha$ protein.

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

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GRANTS

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES