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

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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.

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 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 amplitude of 0 phase depolarization; APD50, AP 50% repolarization; APD90, AP 90% repolarization; Pmax, maximal isometric tension; Pd/dt, velocity of tension development. *P < 0.05 and †P < 0.001 vs. control values.

Western blot. Western blot analysis was used to determine the Gα subunit protein expression level 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 Gα proteins, SDS gel electrophoresis of polypeptides was performed on 8% polyacrylamide gels. Samples, each containing 30 μg 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 blocking solution and incubated with TBS that contained 5% nonfat dry milk (blocking buffer), a procedure that blocks the nonspecific protein binding sites on nitrocellulose. The Gα 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 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). The autoradiograph was scanned with a laser densitometer (NIH Image; Bethesda, MD). The G protein, were added with 10 μl rough membrane at a proper concentration. Nonspecific binding was determined in the presence of 50 μl 10⁻⁴ M propranolol. Reactions were allowed to proceed for 20 min in a 37°C water bath and terminated by adding 7 μl ice-cold Tris-HCl buffer (10 mM Tris-HCl, pH 7.4); the mixture was filtered by glass fiber filters. The filters were washed two times with 7 ml ice-cold Tris-HCl buffer and then dried. Bound radioactivity was measured using an auto gamma-counter. Binding data were analyzed by using nonlinear regression and Scatchard analysis (GraphPad Prism Software) on the computer, and thus the dissociation constant (Kd) between the receptor and radioligand and maximal bound capacity (Bmax) could be obtained.

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

<table>
<thead>
<tr>
<th></th>
<th>Action Potential</th>
<th>Contraction</th>
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<tbody>
<tr>
<td></td>
<td>RP, mV</td>
<td>OS, mV</td>
</tr>
<tr>
<td>Con</td>
<td>-80.7 ± 1.1</td>
<td>11.0 ± 0.8</td>
</tr>
<tr>
<td>CIHH14</td>
<td>-80.6 ± 1.4</td>
<td>11.3 ± 1.5</td>
</tr>
<tr>
<td>CIHH28</td>
<td>-80.6 ± 1.3</td>
<td>11.2 ± 2.1</td>
</tr>
<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; Pd/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.

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$_{50}$ 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 $V_{\text{max}}$ 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.

![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.](http://ajpheart.physiology.org/)

![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.](http://ajpheart.physiology.org/)
the expression level of Gs protein in right ventricular myocardium was 0.26 ± 0.08 (P > 0.05, n = 4), 0.10 ± 0.02 (P < 0.05, n = 4), and 0.14 ± 0.02 (P < 0.05, n = 4) in CIHH14, CIHH28, and CIHH42 rats, respectively. It was lower than 0.32 ± 0.07 (n = 4) in Con rats (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 significantly 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 Gs 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, \(\beta\)-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 chronical 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://www.ajpheart.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://www.ajpheart.org/)
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 (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 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.

REFERENCES