Chronic intermittent hypobaric hypoxia decreases \( \beta \)-adrenoceptor activity in right ventricular papillary muscle

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Chronic intermittent hypobaric hypoxia (CIHH) has an effective cardioprotection against ischemia-reperfusion injury. However, the underlying mechanisms are not fully known. It has been shown that blockade of \( \beta \)-adrenergic receptor exerts anti-arrhythmic action and improves cardiac remodeling in ischemic myocardium. Thus we determined the influence of CIHH on \( \beta \)-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 \( \beta \)-adrenergic receptor with \( \beta \)-iso-proterenol dose-dependently increased action potential duration and the contractility in right ventricular papillary muscle. In CIHH rats, the prolonged effect of \( \beta \)-iso-proterenol 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 \( \beta \)-adrenergic receptor in right ventricular myocardium was significantly lower in CIHH rats. In addition, Western blot analysis revealed that the membrane-bound G protein \( G_\beta \alpha \) expression level in cardiac myocardium was significantly lower in CIHH rats than that in control rats. Collectively, these data suggest that CIHH suppresses \( \beta \)-adrenergic receptor action in right ventricular papillary muscle through decreasing receptor density and affinity, as well as membrane-bound \( G_\beta \alpha \). This mechanism may be involved in the cardiac protective effect of CIHH.

\begin{align*}
\text{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 Ca}\textsuperscript{2+} \text{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\textsuperscript{+} 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.}
\end{align*}

The sympathetic nervous system is critically involved in the regulation of cardiac function through \( \beta \)-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 \( \beta \)-adrenergic receptor blockade could improve cardiac remodeling and have an anti-arrhythmic effect in ischemic myocardium. Also, the activity of \( \beta \)-adrenergic receptor can be changed during hypoxia (12, 13, 15, 16, 19). Little is known, however, whether \( \beta \)-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 \( \beta \)-adrenergic receptor in myocardium and underlying the mechanism. We hypothesized that decreased \( \beta \)-adrenergic receptor function contributes to the cardiac protection produced by CIHH treatment.

\begin{align*}
\text{MATERIALS AND METHODS}
\end{align*}

\textbf{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 (\( \text{Po}_2 = 404 \text{ mmHg}, \text{Po}_2 = 84 \text{ mmHg} \)) 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.

\textbf{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% O\textsubscript{2}. The Tyrode’s solution contained (in mmol/l): 136.8 NaCl, 5.4 KCl, 1.05 MgCl\textsubscript{2}, 1.2 NaHCO\textsubscript{3}, 11.0 glucose, and 5.0 Tris (pH 7.4 ± 0.05, gassed with 100% O\textsubscript{2}). 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 (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.

\textbf{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
Effects of CIHH on action potential and contraction in right ventricular papillary muscle of rats

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>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 animals in each group. RP, resting potential; OS, overshooting; APA, amplitude of action potential (AP); V_{max}, maximal rate of 0 phase depolarization; APD_{50}, AP 50% repolarization; APD_{90}, AP 90% repolarization; P_{max}, maximal isometric tension; P_{Tmax}, 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 $31.5 \pm 6.5$, $34.8 \pm 7.4$ ($P < 0.01$), 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$_{90}$ 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 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 $V_{\text{max}}$ in a dose-dependent manner. Furthermore, APD$_{50}$ and APD$_{90}$ in right papillary muscle were significantly prolonged. However, dl-isoproterenol-induced alterations of action po-

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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.

**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.
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 \( (P < 0.01, n = 4) \), 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 \( \pm \) 12.4 fmol/mg (\( n = 6 \)) in Con rats. Meanwhile, we found the affinity \( (K_d) \) of \( \beta \)-adrenergic receptor in right ventricular myocardium was 344.4 \( \pm \) 150.4 \( (P < 0.05, n = 4) \) and 427.6 \( \pm \) 148.9 \( (P > 0.05, n = 4) \) pM in CIHH28 and CIHH42 rats, respectively. These values were lower than 767.2 \( \pm \) 234.5 pM (\( n = 6 \)) in Con rats. However, adaptation to CIHH, the affinity of \( \beta \)-adrenergic receptor in right ventricular myocardium was significantly attenuated in CIHH rats. In addition, we found the density and affinity of \( \beta \)-adrenergic receptor and expression level of \( G_\text{s} \) protein \( \alpha \)-subunits in Con rats (Fig. 6).

**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 was decreased significantly in CIHH rats. Compared with Con rats, the expression level of \( G_\alpha \) protein in right ventricular myocardium was 0.26 \( \pm \) 0.08 \( (P > 0.05, n = 4) \), 0.10 \( \pm \) 0.02 \( (P < 0.05, n = 4) \), and 0.14 \( \pm \) 0.02 \( (P < 0.05, n = 4) \) in CIHH14, CIHH28, and CIHH42 rats, respectively. It was lower than 0.32 \( \pm \) 0.07 \( (n = 4) \) in Con rats (Fig. 4).

**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 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 \( \beta \)-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...
functional activity of myocardial G_{α} 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_{α} (45 kDa), was reduced in myocardium in CIHH rats, suggesting that the reduced activity of β-adrenergic receptor was related with the reduction of G_{α} 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 G_{α} in right ventricle of rats. These alterations of β-adrenergic receptor may contribute to cardiac protection in CIHH rats.

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