

Inhibition of Carotid Sinus Baroreflex in Neonatal Rats Exposed to Chronic Intermittent Hypobaric Hypoxia

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Abstract

Chronic intermittent hypobaric hypoxia (CIHH) facilitates carotid sinus baroreflex (CSB) in adult rats, but the effect of CIHH on CSB in young rats is not known. The purpose of present study was to investigate the effect of CIHH on CSB in the young rat treated with CIHH from neonatal age, and the role of nitric oxide (NO) and Ca²⁺ in the effect of CIHH. Neonatal male Sprague-Dawley rats were randomly divided into three groups: 42-day CIHH treatment group (CIHH42), 56-day CIHH treatment group (CIHH56), and an age-matched control group (control). CIHH neonatal rats with the maternal rats were exposed to a simulated high-altitude hypoxia in a hypobaric chamber mimicking 5,000-m altitude (O₂ at 11.1%) for 42 or 56 days, 6 h per day, respectively. Isolated carotid sinus perfusion technique was used to test CSB of the rats. After 42-day and 56-day CIHH exposure, the CSB of the rats was inhibited significantly, manifesting as decrease of peak slope (PS) and reflex decrease (RD), and increase of threshold pressure (TP), equilibrium pressure (EP) and saturation pressure (SP). This inhibitory effect was canceled by L-type calcium channel activator Bay K 8644, but not by nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME). The data showed that CIHH inhibited CSB in anesthetized young rats through blocking L-type calcium channels in carotid sinus baroreceptor.

Key Words: baroreflex, calcium channel, carotid sinus, chronic intermittent hypobaric hypoxia, young rat

Introduction

Cumulating evidence has demonstrated that chronic intermittent hypobaric hypoxia (CIHH) has protective effects on the heart against ischemia and reperfusion injury (I/R), promoting recovery of cardiac function, limiting infarct area, and antagonizing arrhythmia during I/R (6, 19, 23, 25). It is well known that the effects of hypoxia on the cardiovascular function critically depend on the experiment protocols, such as cycle length of hypoxia, number of hy-

poxic episodes per day, number of exposure days, and the degree or/and duration of hypoxic exposure (1, 26). Up to now, most studies on CIHH cardioprotection were carried out in adult animals and the effect of CIHH on developing heart is not well defined. Our previous study showed that CIHH treatment (simulating 5,000-m high altitude, 6 h per day for 28 days) produced cardiac protection in adult rats, but generated heart damage in young rats (21), which suggests that the effect of CIHH on the heart is affected not only by CIHH protocols but also by the age of the animals.

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It was reported that CIHH had a depression effect in hypertension patients or in spontaneous hypertensive rats (3, 8). Our previous study showed that CIHH simulating 5,000-m high-altitude for 28 days, 6 h per day did not alter systemic arterial blood pressure (SABP) under normoxic condition, but prevented the decrease of SABP during acute hypoxia (22). It is well known that carotid sinus baroreflex (CSB) plays a key role for the homeostasis of SABP and is modulated by numerous factors, such as nitric oxide (NO) and Ca^{2+} (14, 17). Our recent work showed that CIHH (simulating 5,000-m high-altitude for 28 days, 6 h per day) facilitated CSB in adult rats, which might be one of mechanisms for CIHH modulation on SABP in adult rats. However, the effect of CIHH on young rats is not elucidated. Based on our previous result that the same protocol of CIHH inducing cardioprotection in adult rats but causing cardio-damage in young rats, we proposed a hypothesis that CIHH (simulating 5,000-m high-altitude, 6 h per day) could produce an inhibitory effect on CSB in young rats. And this inhibition of CIHH on CSB might be related to NO and Ca^{2+} in carotid sinus area. In this study, isolated carotid sinus perfusion technique was used to test the effect of CIHH on CSB in young rats. The result showed that CSB was inhibited in CIHH-treated young rats.

Materials and Methods

Animal Grouping and CIHH Treatment

Neonatal male Sprague-Dawley rats, provided by the Experimental Animal Center of Hebei Province, were randomly divided into three groups ($n = 6$ for each group): 42-day CIHH treatment group (CIHH42), 56-day CIHH treatment group (CIHH56), and an age-matched control group. All experiments were conducted in compliance with the guide for the Care and Use of Laboratory Animals (National Research Council, PRC, 1996) and was reviewed and approved by the Ethics Committee for the Use of Experimental Animals at Hebei Medical University. The CIHH rats were exposed to simulated high-altitude hypoxia in a hypobaric chamber mimicking 5,000-m altitude (O_2 : 11.1%) for 42 and 56 days, 6 h per day, respectively. The control animals lived in the same environment as the CIHH animals with free access to food and water except that they breathed normal room air.

Perfusion of Isolated Carotid Sinus

The rats were submitted to CIHH at their neonatal age and the CSB was evaluated after 42 or 56 days CIHH treatment. All the animals were examined within three days after the last hypoxic exposure. Rats were

anaesthetized with urethane (1.0 g/kg, i.p.) and the trachea was cannulated for artificial ventilation. Body temperature of the rats was maintained at 37–38°C throughout the experiment. Perfusion of isolated carotid sinus area was carried out with a method modified by our laboratory (24). Carotid sinus areas were fully exposed by turning the trachea and esophagus in the rostral direction. The sternohyoideus muscles and superior laryngeal nerves were cut. All the bilateral aortic nerves, cervical sympathetic nerves, recurrent laryngeal nerves and the right carotid sinus nerve were sectioned. The common, external and internal carotid arteries and smaller arteries originating from these vessels were exposed and ligated, while carefully leaving the left carotid sinus nerve undisturbed. The occipital artery at its origin from the external carotid sinus was ligated to exclude chemoreceptor effects from the isolated carotid sinus thereby preventing chemoreceptor activation secondary to decrease in carotid sinus pressure. A plastic catheter inserted anterogradely into the left common carotid artery, serving as inlet tube, and another plastic catheter inserted retrogradely into the external carotid artery to serve as outlet tube. The carotid sinus was then perfused with 37°C oxygenated modified Krebs-Henseleit (K-H) buffer (in mmol/L: NaCl 118.0, KCl 4.7, CaCl_2 2.5, MgSO_4 1.6, KH_2PO_4 1.2, NaHCO_3 25, glucose 5.6, pH 7.35–7.45) saturated with 95% O_2 and 5% CO_2 .

Recording of Carotid Sinus Baroreceptor Reflex

The left femoral artery was cannulated for mean arterial blood pressure (MAP) recording with a transducer (YP200, Beijing Xinhang Co. Ltd.) After perfusion of the left carotid sinus with the K-H solution, intrasinus pressure (ISP) was kept at 100 mmHg for 20 min and then was lowered to 0 mmHg rapidly, from which ISP was elevated to 250 mmHg *via* a pulsatile ramp through regulating the speed of peristaltic pump, which was automatically controlled by a program designed by our laboratory (20). It took 0.5 min for ISP to be increased from 0 to 250 mmHg. The process was repeated at an interval of 5 min to check the stability of the baroreflex.

By perfusing the left carotid sinus with the K-H solution and elevating the ISP, a functional curve for the ISP-MAP relation was constructed, and the functional parameters of baroreflex, such as threshold pressure (TP), equilibrium pressure (EP), saturation pressure (SP), operating range (OR), peak slope (PS) and reflex decrease of MAP (RD) were determined. TP was the ISP at which MAP decreased 5 mmHg in response to the increase of ISP. SP was the ISP at which MAP just showed no further reflex decreasing with an increase in ISP. EP was the ISP that equaled to systemic MAP. OR was calculated through SP minus TP. When PS

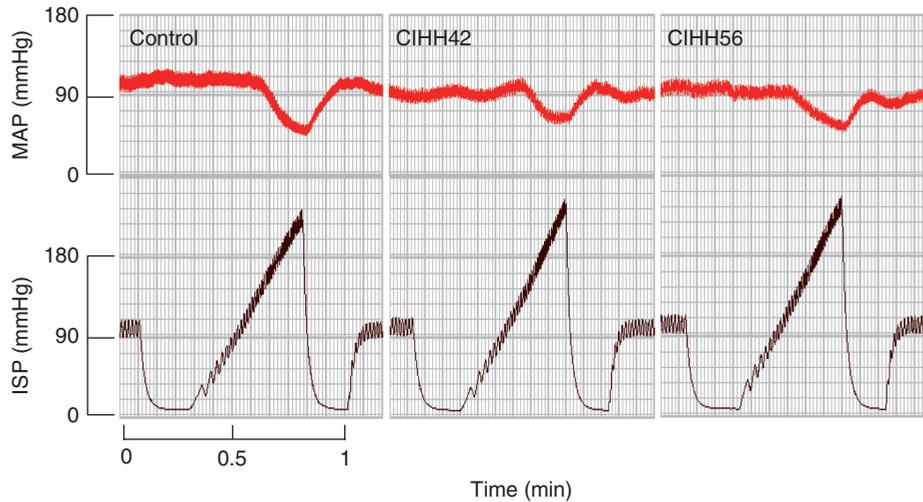


Fig. 1. Original recording showing the reflex response of CIHH on carotid sinus baroreflex activity in young rats. Control: control group; CIHH42: 42-day chronic intermittent hypobaric hypoxia (CIHH) treatment group; CIHH56: 56-day CIHH treatment group. ISP: intrasinus pressure; MAP: mean arterial pressure.

and RD decrease, and TP, EP and SP increase, it means that CSB is inhibited or injured. That is to say, CSB has become more insensitive to detect deflection of artery blood pressure, or more ineffective to antagonizing fluctuations in artery blood pressure.

The functional curve and functional parameters were obtained after perfusing carotid sinus of each group of animals with the K-H solution. Data for the ISP-MAP relationships were collected and fitted to a sigmoidal logistic function curve, the baroreceptor function curve. The baroreflex gain was calculated as the ratio of change in MAP to the change in ISP ($\Delta\text{MAP}/\Delta\text{ISP}$, expressed as mmHg/mmHg), which was considered to be the marker of the baroreceptor reflex sensitivity. Subsequently, Bay K 8644 (500 nM), an L-type calcium channel agonist, or *L*-NAME (100 μM), a nitric oxide synthase (NOS) inhibitor, was added into the K-H solution according to the protocols, respectively. Baroreflex parameters were obtained after treatment with Bay K 8644 or *L*-NAME. At the end of experiment, the animals were sacrificed by an over dose of urethane (3.0 g/kg, i.v.).

Drugs

Bay K 8644 and *L*-NAME were purchased from Sigma Co. (St. Louis, MO, USA). Bay K 8644 was dissolved in 99% ethyl alcohol. The final concentration of ethyl alcohol in the K-H solution was 0.05%, at which no change of CSB was observed. *L*-NAME was dissolved in distilled water.

Data Analysis

All data were expressed as mean \pm SD. One-way

analysis of variance (ANOVA) and Student-Newman-Keuls test were applied for comparison between control and CIHH groups. Paired *t*-test was used to compare the effect before and after drug administration. $P < 0.05$ was considered statistically significant.

Results

Effect of CIHH on CSB in Young Rats

During perfusing the carotid sinus with K-H solution and elevating ISP from 0 to 250 mmHg, the reflex decrease amplitude of MAP was decreased markedly in CIHH42 and CIHH56 rats (Fig. 1). PS and RD rats decreased, while TP, EP and SP increased in CIHH42 and CIHH56 rats compared with the control rats ($P < 0.05$, Table 1). But there was no difference of parameters between CIHH42 and CIHH56 rats. The functional curve of CSB shifted rightward and upward, and the gain curve of the baroreflex shifted downward in the CIHH42 and CIHH56 rats compared with control rats. The results suggest that CIHH treatment inhibits CSB in young rats in a time-independent manner.

Influence of Bay K 8644 on Inhibition of CSB Induced by CIHH in Young Rats

In order to investigate whether activity of L-type calcium channel involved in the inhibitory effect of CIHH on CSB in the young rat, L-type calcium channel opener Bay K 8644 was used. Perfusing the isolated carotid sinus with K-H solution containing Bay K 8644 (500 nM) for 20 min, there were no changes of functional parameters of CSB in the control rat. On the

Table 1. Effects of CIHH on the functional parameters of carotid sinus baroreceptor in anesthetized young rats

	TP/mmHg	EP/mmHg	SP/mmHg	OR/mmHg	PS	RD/mmHg
Control	63.72 ± 1.61	92.36 ± 1.08	173.30 ± 1.86	109.55 ± 1.70	0.42 ± 0.03	42.66 ± 2.76
CIHH42	72.64 ± 3.04**	94.50 ± 1.56*	181.94 ± 4.18**	108.67 ± 3.62	0.35 ± 0.01**	36.64 ± 1.48**
CIHH56	71.64 ± 3.60**	93.99 ± 1.60*	181.56 ± 5.23**	109.93 ± 3.44	0.35 ± 0.02**	36.36 ± 2.12**

The data was expressed as Mean ± SD, n = 6 for each group. TP: threshold pressure; EP: equilibrium pressure; SP: saturation pressure; OR: operation range; PS: speak slope; RD: reflex decrease in MAP. Control: control group; CIHH42: 42-day chronic intermittent hypobaric hypoxia (CIHH) treatment group; CIHH56: 56-day CIHH treatment group. * $P < 0.05$, ** $P < 0.01$ vs. Control.

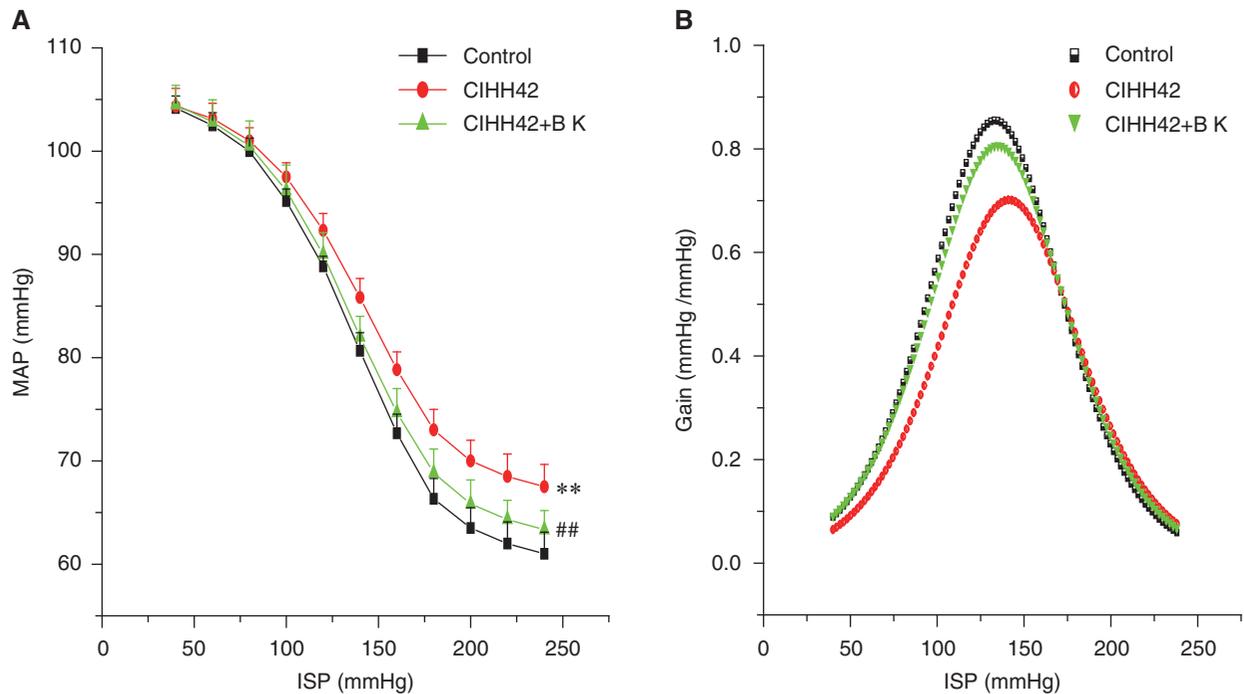


Fig. 2. Changes of carotid sinus baroreflex after Bay K 8644 administration in anesthetized CIHH42 young rats. A. Functional curve of baroreflex. B. Baroreflex gain. Control: control group; CIHH42: 42-day chronic intermittent hypobaric hypoxia (CIHH) treatment group. ISP: intrasinus pressure; MAP: mean arterial pressure. B K: Bay K 8644. The data was expressed as mean ± SD, n = 6 for each group. ** $P < 0.01$ vs. Control, ### $P < 0.01$ vs. CIHH42.

contrary, the change of functional parameters of CSB in the CIHH rats, including the functional curve of CSB and gain curve of CSB, was abolished (Figs. 2 and 3), which suggests that the inhibitory effect of CIHH on CSB in young rats is related to L-type calcium channel in carotid sinus.

Influence of L-NAME on Inhibition of Carotid Sinus Baroreflex Induced by CIHH in Young Rats

In order to investigate whether NO is involved in the inhibitory effect of CIHH on CSB in the young rat, the NOS inhibitor L-NAME was used. Perfusing the isolated carotid sinus with K-H solution containing L-NAME (100 μ M) for 20 min, there was no change on CSB in both the control and the CIHH rats (Table

2), which suggests that the inhibitory effect of CIHH on CSB in young rats is not related to NO in carotid sinus.

Discussion

The present study was designed to directly observe CSB in young rats after 42 or 56 days CIHH treatment (simulating 5,000-m altitude, 6 h per day) from neonatal age by using the technique of perfusing isolated carotid sinus. The results showed that the functional curve of CSB was shifted rightward and upward, the baroreflex parameters changed obviously, such as PS and RD decreased and TP increased, and the gain of baroreflex decreased in CIHH treated young rats compared with the control rats. And all

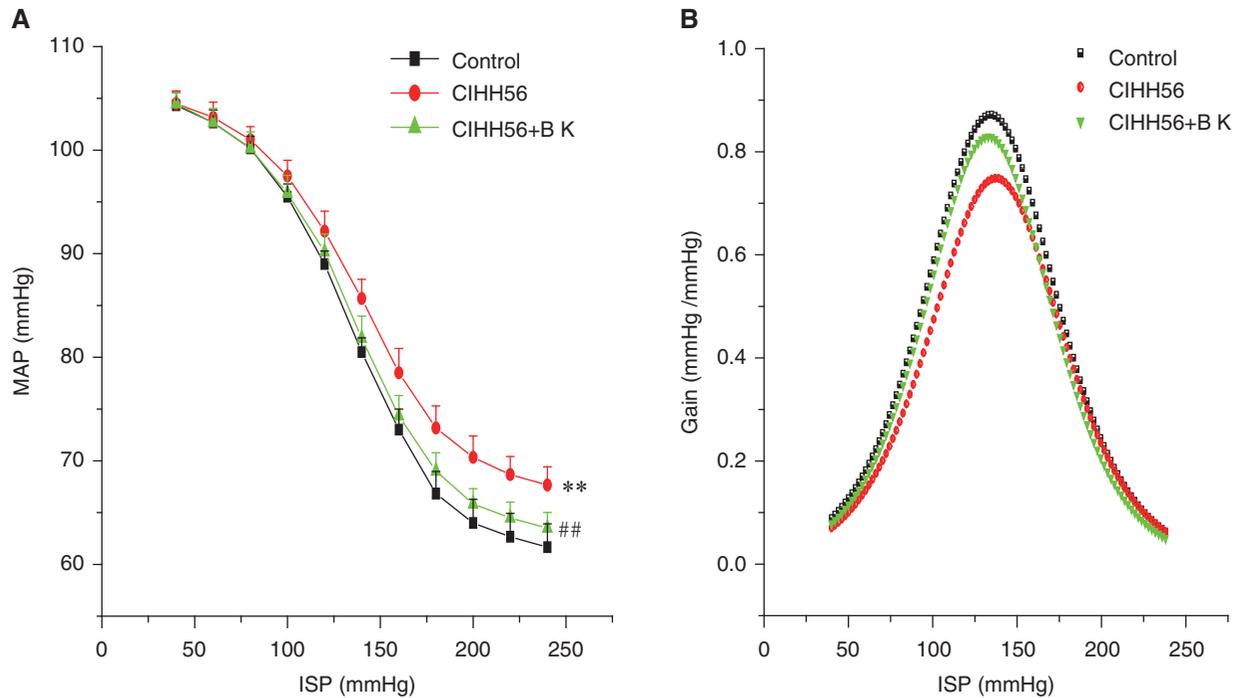


Fig. 3. Changes of carotid sinus baroreflex after Bay K 8644 administration in anesthetized CIHH56 young rats. A. Functional curve of baroreflex. B. Baroreflex gain. Control: control group; CIHH56: 56-day chronic intermittent hypobaric hypoxia (CIHH) treatment group. ISP: intrasinus pressure; MAP: mean arterial pressure. B K: Bay K 8644. The data was expressed as mean \pm SD, $n = 6$ for each group. $**P < 0.01$ vs. Control, $##P < 0.01$ vs. CIHH56.

Table 2. Effect of L-NAME on function parameters of carotid sinus baroreceptor in anesthetized young rats

	TP/mmHg	EP/mmHg	SP/mmHg	OR/mmHg	PS	RD/mmHg
Control	63.22 \pm 1.42	92.36 \pm 1.27	173.77 \pm 1.91	110.55 \pm 1.56	0.44 \pm 0.03	43.74 \pm 2.88
Control + L-NAME	63.72 \pm 1.77	92.70 \pm 1.04	172.93 \pm 1.85	109.21 \pm 1.76	0.43 \pm 0.03	43.04 \pm 2.52
CIHH42	71.42 \pm 4.33**	94.88 \pm 1.05*	181.64 \pm 3.89**	110.22 \pm 2.89	0.36 \pm 0.02**	36.50 \pm 1.79**
CIHH42+L-NAME	72.43 \pm 4.56	94.54 \pm 1.48	180.80 \pm 4.71	108.38 \pm 3.67	0.36 \pm 0.02	36.96 \pm 1.88
CIHH56	71.09 \pm 4.16**	94.54 \pm 1.35*	180.47 \pm 5.43*	109.38 \pm 3.64	0.36 \pm 0.03**	36.69 \pm 2.63**
CIHH56+L-NAME	72.43 \pm 5.17	94.37 \pm 2.95	181.30 \pm 5.42	108.71 \pm 1.96	0.35 \pm 0.03	35.57 \pm 3.36

See to Table 1. $*P < 0.05$, $**P < 0.01$ vs. Control.

CIHH-induced changes of CSB were eliminated by Bay K 8644, an agonist of L-type calcium channel. The results demonstrate for the first time that CIHH inhibits CSB in the young rat and the inhibitory effect of CIHH on CSB is related to the blocking of L-type calcium channel in carotid sinus.

Our present study displayed an inhibitory effect of CIHH (simulated 5,000-m, 6 h per day for 42 or 56 days) on CSB in young rats. This result is contradictory to that of our previous study in adult rats, in which CSB was facilitated after rats exposed to CIHH simulating 5,000-m high-altitude for 28 days, 6 h per day (7). The results demonstrated that same level of CIHH may produce totally different effect, facilitation of CSB in adult animals but inhibition of CSB in young

animals. It was reported that antenatal exposure to intermittent hypoxia resulted in impaired physical development of all offspring during the early 15-day postnatal period and caused changes in the vegetative balance of heart regulation (15). It was also reported that a carotid body response to long-term changes in environmental oxygen was different between neonates and adults (11). In agreement with the hypothesis, this study showed that CIHH simulating 5,000-m altitude for 42 or 56 days, 6 h per day produced an inhibitory effect on CSB in young rats. Taken together, the results suggest that age of the animal, at least in part, is an important factor of determining the effects of CIHH on CSB.

The result of this study confirmed our hypothesis

that CIHH simulating 5,000-m high altitude for 6 h per day would lead to damage CSB in young rats. Our data also demonstrated that there was a similarity of CIHH effect on the heart and CSB in both adult and young rats. It is known that biological effects of intermittent hypoxia are highly dependent on the level, duration and protocol of the hypoxia treatment (2). Based on our previous and present researches, it is suggested that the protocol or intensity of CIHH is another important factor determining the effect of CIHH on CSB in rats.

It has been demonstrated that baroreceptor of carotid sinus can be activated by stretching of carotid sinus wall *via* increasing calcium influx through stretch-activated channels in baroreceptor neurons (5). Studies have shown that ginkgolide B can inhibit CSB through suppressing the stretch-activated channels (16). Moreover, Liu *et al.* reported that the inhibitory action of rhynchophylline on carotid sinus baroreceptor activity is ascribed to the blocking of calcium influx (9). In this study, the inhibitory effect of CIHH on CSB in young rats was totally cancelled by L-type calcium channel opener Bay K 8644, suggesting that the observed inhibitory effect of CIHH on CSB in young rats was a consequence of blocking the L-type calcium channel and inhibiting calcium influx in carotid sinus baroreceptor. The mechanism of the CIHH effect on L-type calcium channel and calcium ions in carotid sinus baroreceptor needs further investigation.

Nitric oxide (NO), widely present in the body, is an important chemical messenger generated from L-arginine by the activity of NOS (12). It has been reported that NOS-positive neurons exist in the carotid sinus in guinea pigs and rats (4). Furthermore, NOS has been found in the modulation pathway of baroreflex (10), implying that NO can be synthesized in carotid sinus and involved in regulation of baroreflex. Available data on the effects of hypoxia on NO and/or NOS are controversial (13, 18), which probably because different intermittent hypoxia protocols, organs and/or cells and assay methods were used. In this study, however, pre-treatment with L-NAME, an inhibitor of NOS, did not alter the inhibitory effect of CIHH on CSB, which suggests that NO is unnecessary in the inhibitory effect of CIHH on CSB.

In conclusion, CIHH treatment simulating 5,000-m high altitude from neonatal age inhibits CSB in young rats, which might be related to the blocking of L-type calcium channels in carotid sinus baroreceptor.

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