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Facilitation of Chronic Intermittent Hypobaric Hypoxia on Carotid Sinus Baroreflex in Anesthetized Rats

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Abstract

Our previous study showed that chronic intermittent hypobaric hypoxia (CIHH) could prevent decreases in systemic arterial blood pressure (SABP) during acute hypoxia. However, the mechanism was not clear. The purpose of the present study was to observe whether the carotid sinus baroreflex (CSB) was involved in the antagonizing effect of CIHH on SABP decrease induced by acute hypoxia and to explore the underlying mechanism using perfusion technique in rat isolated carotid sinus area. After 14-day and 28-day CIHH exposure, the CSB in rats was enhanced markedly, manifesting as increases in peak slope and reflex decrease of MAP, and decreases in threshold pressure and saturation pressure. This facilitation of CSB was partly abolished by Glibenclamide (Gli, 10 μM), an ATP sensitive potassium (K_{ATP}) channel blocker, but was not influenced by *L*-NAME (100 μM), a nitric oxide synthase inhibitor. The results of the study suggested that CIHH facilitated CSB through opening the K_{ATP} channels in carotid sinus of anesthetic rats and might be one of the mechanisms of CIHH keeping SABP homeostasis during acute hypoxia.

Key Words: chronic intermittent hypobaric hypoxia, carotid sinus, baroreflex, mean arterial pressure, K_{ATP} channel, rat

Introduction

In recent years, chronic intermittent hypobaric hypoxia (CIHH) has been proved to have a cardio-protective effect against ischemia/hypoxia injury (9, 13). Our previous work showed that rats given 28 days of CIHH treatment, mimicking 5,000 m altitude, 6 h per day, displayed an obvious cardiac protection, including the prevention of arrhythmia induced by ischemia/reperfusion, increasing of antioxidant (26), reducing infarct size and limiting the contractile falling in ischemia/reperfusion heart of the rat (28). Further studies indicated that cardiac protection induced by CIHH treatment might be related to prolongation of action potential duration (APD), opening of ATP-sensitive potassium (K_{ATP}) channel, increasing of capillary density and coronary blood flow as well as increasing of expression of heat shock protein (HSP) (25, 27-29).

It is known that systemic arterial pressure is frequently elevated in patients with obstructive sleep apnea syndrome (OSAS) (8) in which intermittent hypoxia is experienced. On the contrary, it was reported by former Soviet scientists that CIHH had a depression effect in hypertension patients or spontaneously hypertensive rats (3, 6). Our previous study (24) showed that the CIHH simulated 5,000 m high-altitude (6 h per day for 28 days) could not alter systemic arterial blood pressure (SABP) under normoxic condition, but prevented the decrease of

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SABP during acute hypoxia. However, the underlying mechanism was not clear.

It is well known that carotid sinus baroreflex (CSB) plays a key role in the rapid regulation of SABP, but it is not known whether the CSB was involved in the antagonizing effect of CIHH on SABP decrease induced by acute hypoxia. The purpose of the present study is to investigate the effect of CIHH on CSB using a perfusion technique in isolated carotid sinus area of the rat and to explore the underlying mechanism.

Materials and Methods

Animal Group and CIHH Exposure

Male Sprague-Dawley rats, provided by the Experimental Animal Center of Hebei Province (Grade II,Certificate No702016), originally weighing 200-240 g and finally weighing 320-360 g, were randomly divided into three groups: Age-matched control group (Con), 14-day chronic intermittent hypobaric hypoxia treatment group (CIHH14) and 28-day chronic intermittent hypobaric hypoxia treatment group (CIHH28). 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 a simulated high-altitude hypoxia in a hypobaric chamber mimicking 5,000 m altitude (O_2: 11.1%) for 14 and 28 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. The body weights of the rats were recorded each week.

Arterial Blood Pressure (ABP) and Blood-Gas Measurement

ABP was measured weekly in conscious rats with a manometer manufactured by Powerlab/8SP data acquisition system (AD Instrument, Castle Hill, Australia) using the tail-cuff method (12). ABP was measured at least three times for each animal. To avoid variations in blood pressure due to day cycle, all measurements were carried out between 17:00 and 19:00. Blood-gas measurements were carried out on each group. Arterial and venous blood samples were withdrawn from the right femoral artery and vein before perfusion. Arterial and venous partial pressures of oxygen were measured with a blood gas analyzer (MEDICA Easy Blood Gas, Bedford, MA, USA).

Perfusion of Left Isolated Carotid Sinus

Rats were anaesthetized with urethane (1.0 g/kg, i.p.). The trachea was cannulated for ventilation. Body temperature was maintained at 37 ~ 38°C throughout the experiment.

The perfusion of isolated carotid sinus area was carried out with a method modified by our laboratory (21). 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, right carotid sinus nerve, cervical sympathetic nerves and recurrent laryngeal nerves 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. Ligation of the occipital artery at its origin from the external carotid artery excluded chemoreceptor from the isolated carotid sinus thereby preventing chemoreceptor activation secondary to decrease in carotid sinus pressure. Plastic catheter introduced into the left common carotid artery in the anterograde way (served as an inlet tube) was attached to a peristaltic pump which controlled the intrasinus pressure (ISP). ISP was monitored by a pressure transducer (XH YP200) connected with the inlet tube, and the acquired signals were recorded by Powerlab/8SP data acquisition system (AD Instrument, Castle Hill, Australia). Another plastic catheter inserted into the external carotid artery served as an outlet tube. The carotid sinus was then perfused with warm (37°C) oxygenated modified Krebs-Henseleit (K-H) solution (mM: NaCl 118.0, KCl 14.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.6, pH 7.35~7.45) bubbled with 95% O_2 and 5% CO_2.

Recording of Carotid Sinus Baroreceptor Reflex

The left femoral artery was cannulated for recording mean arterial blood pressure (MAP) with a transducer (XH YP200). After perfusion of the left carotid sinus with the K-H solution, ISP was kept at 100 mmHg for 20 min and was then rapidly lowered to 0 mmHg from which ISP was elevated to 250 mmHg *via* a pulsatile ramp by regulating the speed of peristaltic pump, which was automatically controlled by a program designed by our laboratory (23). 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 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.

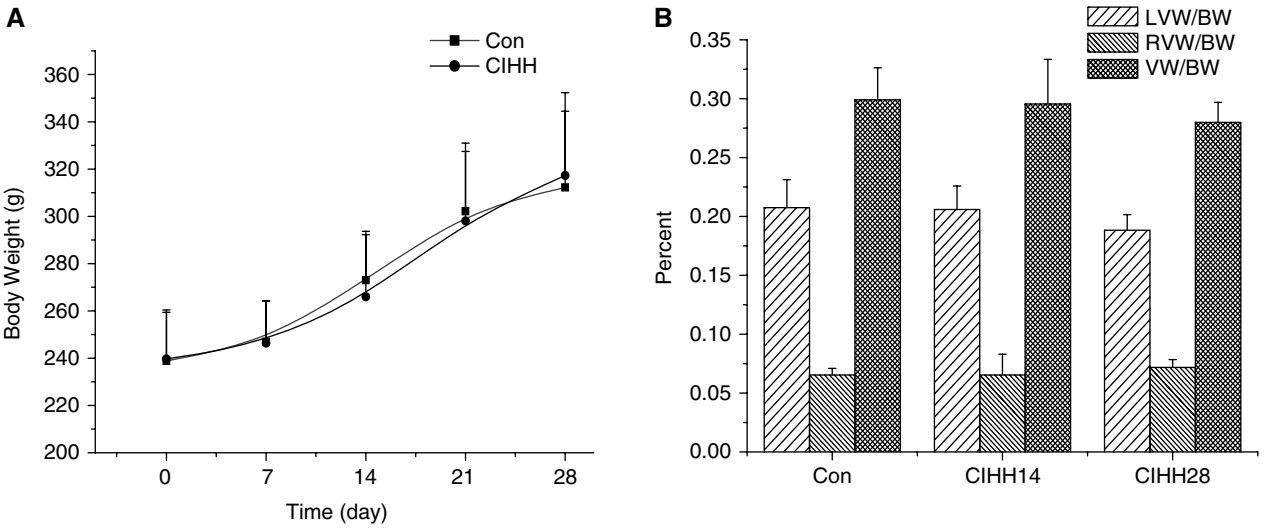

Fig. 1. Effect of chronic intermittent hypobaric hypoxia (CIHH) on body weight and heart weight of rats. A: body weight. n = 15 for each group. Con: Control group; CIHH14: 14-day CIHH treatment group. CIHH28: 28-day CIHH treatment group. B: ventricle weight. n = 7 for each group. BW: body weight; VW: ventricle weight; RVW: right ventricle weight; LVW: left ventricle weight. Data are expressed as means ± SD.

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.

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, which is the baroreceptor function curve. The baroreflex gain was calculated as the ratio of change in MAP to the change in ISP (ΔMAP/ΔISP, expressed as mmHg/mmHg), which was considered to be the marker of the baroreceptor reflex sensitivity. Glibenclamide (Gli, 10 μM), a K_{ATP} channel blocker, or *L*-NAME (100 μM), a nitric oxide synthase (NOS) inhibitor, was then added into the K-H solution according to the protocols, respectively. Baroreflex parameters were obtained after treatment with Gli and *L*-NAME.

At the end of the experiment, the animals were sacrificed by an over-dose of urethane (3.0 g/kg, i.v.). The hearts were removed rapidly and the weights of the heart, the left ventricle with interventricular septum and the right ventricle were measured.

Drugs

Glibenclamide and *L*-NAME were purchased from Sigma Co. Glibenclamide was initially dissolved in dimethylsulfoxide (100 μM). The final concentration of dimethylsulfoxide in the K-H solution was 0.01% (v/v). *L*-NAME was dissolved in distilled water.

Data Analysis

All data were expressed as means ± SD. One-way analysis of variance (ANOVA) and Student-Newman-Keuls test were applied for comparison between 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 Body Weight and Heart Weight

The body weight of rats in the CIHH groups had no significant change compared with the Con rats ($P > 0.05$, Fig. 1A). There were also no significant differences in the ratio of whole ventricle weight to body weight, the ratio of left ventricle weight to body weight and the ratio of right ventricle weight to body weight between CIHH and Con animals, which meant that CIHH treatment under this experimental condition did not result in heart hypertrophy ($P > 0.05$, Fig. 1B).

Effects of CIHH on Blood Gas and ABP

There were no significant differences of blood gas, including PaO_2, PvO_2 and Pa-vO_2 between Con and CIHH rats ($P > 0.05$, Table 1). The ABP in CIHH rats were also unchanged compared with the Con rats

Table 1. Blood gas values in Con and CIHH rats

Groups	PaO_2 (mmHg)	PvO_2 (mmHg)	SaO_2 (%)	Pa-vO_2 (mmHg)
Con	89.0 ± 12.5	47.0 ± 2.8	95.4 ± 1.7	43.0 ± 22.6
CIHH14	90.3 ± 1.4	43.0 ± 7.2	95.5 ± 1.9	42.6 ± 4.8
CIHH28	88.7 ± 11.6	42.3 ± 6.4	93.2 ± 3.8	43.7 ± 10.0

Data are expressed as means ± SD, n = 7 for each group; CIHH: chronic intermittent hypobaric hypoxia; Con: control; CIHH14: CIHH for 14 days; CIHH28: CIHH for 28 days; PaO_2: arterial oxygen partial pressure; PvO_2: venous oxygen partial pressure; Pa-vO_2: arteriovenous oxygen pressures gradient; SaO_2: saturation of arterial blood oxygen.

Table 2. Effects of CIHH on ABP in conscious rats

Groups	n	ABP (mmHg)
Con	7	97.2 ± 8.6
CIHH14	7	97.4 ± 20.3
CIHH28	7	99.8 ± 7.5

Data are expressed as means ± SD. ABP: arterial blood pressure.

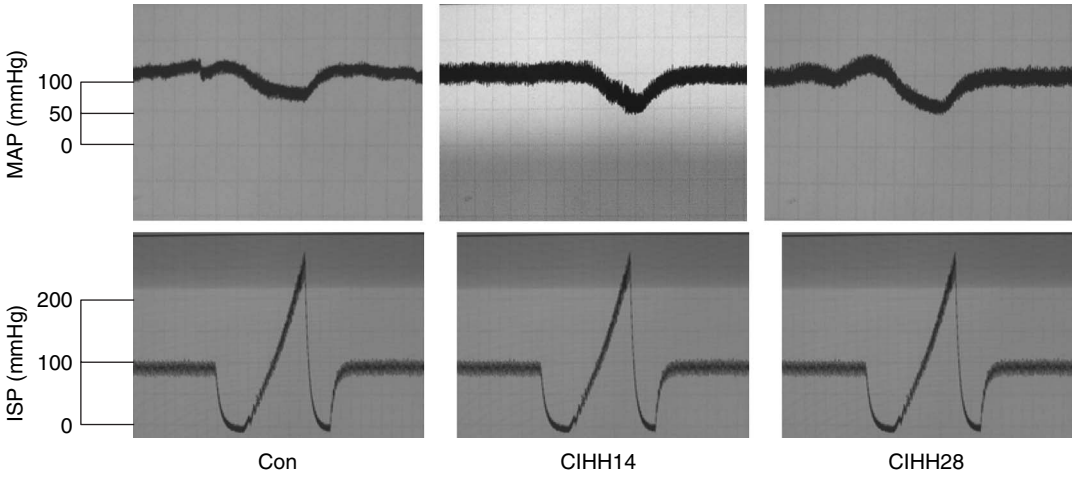

Fig. 2. Original recording showing the reflex response of carotid sinus baroreflex activity in anesthetized rats. Con, CIHH14 and CIHH28 are as defined in the legend to Fig. 1. ISP: intrasinus pressure; MAP: mean arterial pressure.

($P > 0.05$, Table 2).

Effects of CIHH on Carotid Sinus Baroreflex (CSB)

By perfusing the carotid sinus with K-H solution and elevating ISP from 0 to 250 mmHg, the reflex decrease of MAP was increased markedly (Fig. 2). The functional curve of the baroreflex in the CIHH animals shifted leftward and downward in a time-dependent manner (Fig. 3A). PS and RD were increased while TP, EP and SP were decreased in the CIHH rats compared with the Con rats ($P < 0.05$, Table 3). The gain of CSB was increased in the CIHH rats compared with the Con rats (Fig. 3B). The results suggest that CIHH exposure can facilitate CSB of rats.

Effects of Glibenclamide (Gli) on CSB

After perfusing the isolated carotid sinus with K-H solution containing Gli (10 μM) for 10 min, there were no significant changes of functional parameters of CSB in the Con group before and after Gli application. The facilitatory effect of baroreflex induced by CIHH was partially abolished after Gli application in CIHH14 and CIHH28 rats (Table 4, Fig. 4).

Effects of L-*NAME on CSB*

After perfusing the isolated carotid sinus with K-H solution containing *L*-NAME (100 μM) for 10 min, there were no changes of CSB before and after

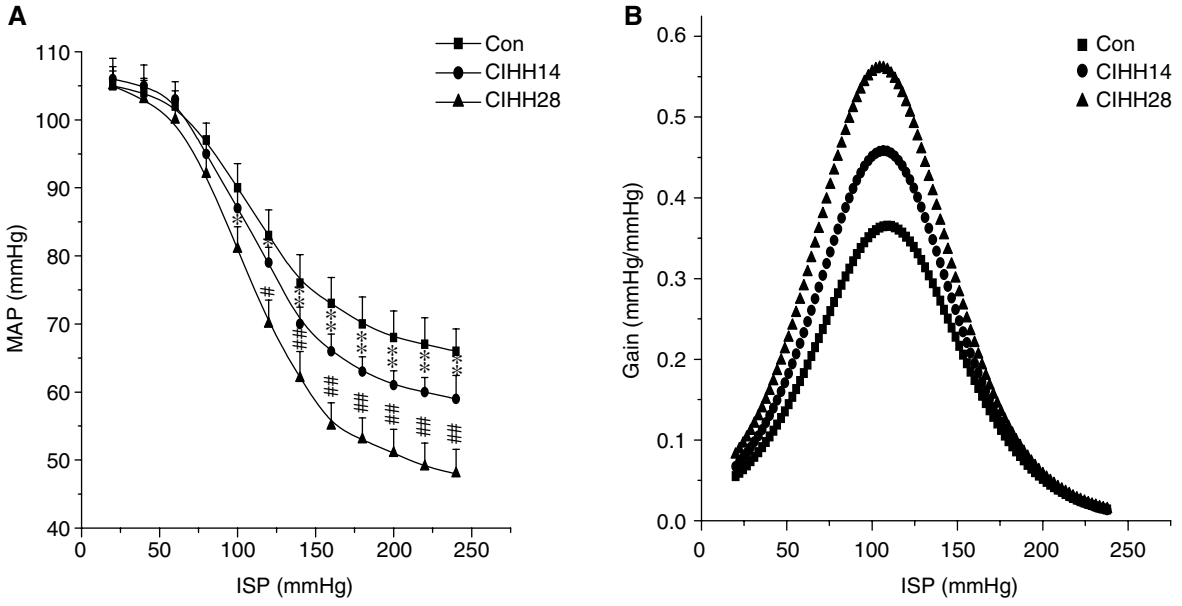

Fig. 3. Effect of CIHH on carotid sinus baroreflex in anesthetized rats. A. Functional curve of baroreflex. B. Baroreflex gain. See legends to Figs. 1 and 2. Data are expressed as means ± SD; n = 6 for each group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with Con; $^{\#\#}P < 0.01$ compared with CIHH14.

Table 3. Effects of CIHH on the functional parameters of carotid sinus baroreflex in anesthetized rats

Groups	TP (mmHg)	EP (mmHg)	SP (mmHg)	OR (mmHg)	PS	RD (mmHg)
Con	65.82 ± 2.89	92.27 ± 3.05	171.96 ± 1.31	106.13 ± 3.38	0.37 ± 0.02	37.28 ± 1.12
CIHH14	59.20 ± 3.02**	88.54 ± 1.49*	167.94 ± 1.79**	107.13 ± 1.65	0.45 ± 0.01**	44.77 ± 1.97**
CIHH28	53.17 ± 2.13**##	86.33 ± 1.49**	164.52 ± 1.50**#	112.18 ± 1.31**##	0.56 ± 0.02**##	57.30 ± 2.47**##

Data are expressed as means ± SD, n = 6 for each group; TP: threshold pressure; EP: equilibrium pressure; SP: saturation pressure; OR: operation range; PS: speak slope; RD: reflex decrease of MAP. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. Con, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs*. CIHH14.

Table 4. Effect of Gli on function parameters of carotid sinus baroreflex in anesthetized rats

Groups	TP (mmHg)	EP (mmHg)	SP (mmHg)	OR (mmHg)	PS	RD (mmHg)
Con	67.24 ± 2.56	94.57 ± 3.04	171.96 ± 1.31	104.52 ± 2.84	0.39 ± 0.03	37.65 ± 2.31
Con+Gli	66.03 ± 3.42	93.03 ± 5.49	170.36 ± 1.35	104.93 ± 2.20	0.38 ± 0.02	38.19 ± 2.21
CIHH14	60.81 ± 2.98**	89.18 ± 2.65*	168.44 ± 1.11**	107.34 ± 1.65	0.45 ± 0.01**	46.89 ± 3.30**
CIHH14 + Gli	64.02 ± 0.71*#	88.74 ± 3.15*	170.55 ± 1.19#	106.53 ± 1.42	0.43 ± 0.01#	42.08 ± 3.08*#
CIHH28	54.37 ± 1.31**##	84.82 ± 2.75**	165.93 ± 0.84**#	111.56 ± 1.05**##	0.55 ± 0.03**##	57.44 ± 2.59**##
CIHH28 + Gil	60.63 ± 1.68**++	87.16 ± 2.49*	168.74 ± 1.23**++	108.14 ± 1.01*++	0.45 ± 0.03**++	45.87 ± 2.98**++

Data are expressed as means ± SD, n = 6 for each group. See footnotes to Tables 1 and 3. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. Con, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs*. CIHH14, $^{++}P < 0.01$ *vs*. CIHH28.

applying *L*-NAME to the Con, CIHH14 and CIHH28 groups (Table 5, Fig. 5). To further confirm the negative effect of *L*-NAME on CSB, we increased the dosage of *L*-NAME to 200 and 400 μM and obtained the same negative results as in the case of using 100 μM *L*-NAME.

Discussion

The present study demonstrated firstly that CIHH treatment could facilitate CSB in rats. Using the perfusing technique in carotid sinus of rats, the present study showed that the functional curve of baroreflex

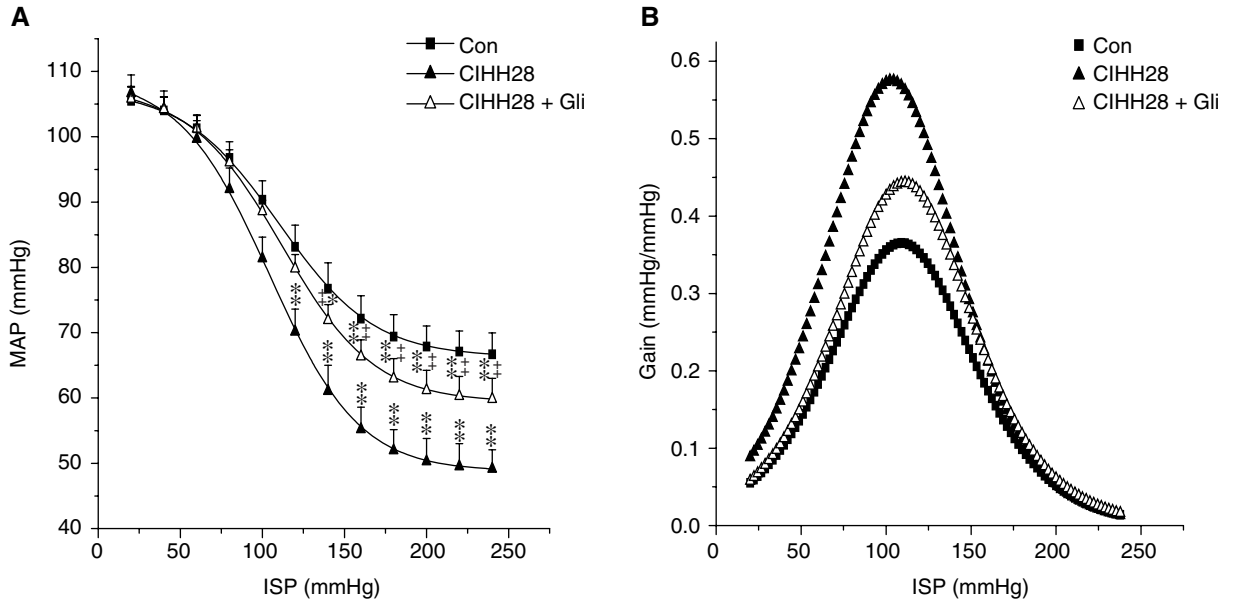

Fig. 4. Change of carotid sinus baroreflex after applying Gli to anesthetized CIHH28 rats. A. Functional curve of baroreflex. B. Baroreflex gain. See legends to Figs. 1 and 2; Gli: Glibenclamide. Data are expressed as means ± SD, n = 6 for each group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with Con; $^{++}P < 0.01$ compared with CIHH28.

Table 5. Effect of *L*-NAME on function parameters of carotid sinus baroreflex in anesthetized rats

Groups	TP (mmHg)	EP (mmHg)	SP (mmHg)	OR (mmHg)	PS	RD (mmHg)
Con	65.63 ± 3.06	93.97 ± 3.15	170.55 ± 1.79	106.33 ± 1.31	0.38 ± 0.02	38.59 ± 1.54
Con + *L*-NAME	66.62 ± 2.62	92.76 ± 2.62	169.95 ± 2.18	105.12 ± 1.15	0.38 ± 0.01	38.78 ± 2.23
CIHH14	60.21 ± 1.22**	89.95 ± 2.08*	169.14 ± 2.62**	107.94 ± 0.89	0.45 ± 0.01**	47.13 ± 1.33**
CIHH14 + *L*-NAME	60.42 ± 1.79	88.94 ± 1.35	169.55 ± 1.31	108.14 ± 0.71	0.45 ± 0.02	47.01 ± 2.08
CIHH28	53.36 ± 1.65**##	87.14 ± 2.17**	164.92 ± 2.40**#	112.76 ± 2.29**##	0.58 ± 0.03**##	58.94 ± 2.81**##
CIHH28 + *L*-NAME	52.54 ± 0.84	86.33 ± 1.42	165.53 ± 2.06	113.16 ± 1.96	0.59 ± 0.03	59.37 ± 3.16

Data are expressed as means ± SD, n = 6 for each group. See footnotes to Tables 1 and 3. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* Con, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* CIHH14.

in CIHH rats shifted leftward and downward, the gain of baroreflex increased compared with the Con rats. The parameters of baroreflex were obviously changed in the CIHH rats, including increases of PS and RD, and decreases of TP and SP. The effect of CIHH on baroreflex was time-dependent, the longer the CIHH exposure, the more obvious the CIHH effect appeared.

The arterial baroreflex is a major negative feedback mechanism for regulation of arterial pressure. Under normal physiological conditions, increases in ABP stretch the wall of the vascular smooth muscle thereby activating the baroreceptors located at the carotid sinus and aortic arch, and the signals of the baroreceptors are transmitted to the central nervous system through afferent nerves, glossopharyngeal and vagus nerves. These signals inhibit the sympathetic outflow to the cardiovascular system, and excite the parasympathetic nerves which reduce peripheral resistance and cardiac output. All of the above effects tend to reduce the blood pressure toward the normal level. On the contrary, decreases of ABP can diminish baroreflex resulting in arterial blood increase toward the normal level. Hence, baroreflex exerts a buffering action against fluctuations in blood pressure and maintains the homeostasis of blood pressure. Our previous study showed that CIHH had no effect on systemic blood pressure under basic physiological conditions, but effectively antagonized the decline of blood pressure caused by severe acute hypoxia (24). The result of the present study displayed that CIHH facilitated the CSB in the rat. The facilitation of CSB means that the buffering effect of CSB is enhanced, or has become more sensitive to detecting deflection of ABP, or more effective to antagonizing fluctuations in ABP. The facilitation effect of CIHH on CSB might be a mechanism for the protective effect of CIHH against the decline of blood pressure induced by severe acute hypoxia.

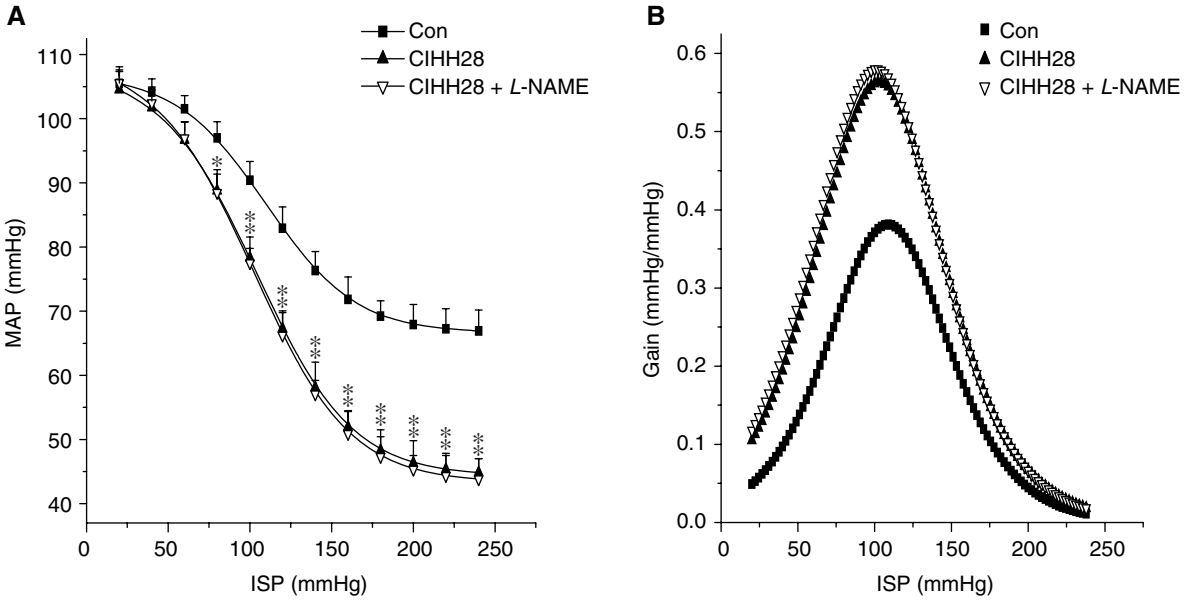

Fig. 5. Change of carotid sinus baroreflex after applying *L*-NAME in anesthetized CIHH28 rats. A. Functional curve of baroreflex. B. Baroreflex gain. See legends to Figs. 1 and 2. Data are expressed as means ± SD; n = 6 for each group. $*P < 0.05$, $**P < 0.01$ compared with Con.

K_{ATP} channels are expressed in most excitable tissues and play a critical role in numerous physiological processes through regulating neuronal activity (1). Activation of K_{ATP} channel generally results in hyperpolarization of the cells. K_{ATP} channel activity is sensitive to changes in the concentrations of both intra- and extra-cellular factors such as ATP, nucleotide diphosphates, molecular oxygen (O_2), hydrogen ions (H^+), adenosine, prostacyclin and NO (14, 15). There are a lot of studies that confirm that the opening of K_{ATP} channels, especially in mitochondria, plays an important role in cardioprotection induced by different strategies such as ischemic pre-conditioning, post-conditioning and chronic hypoxia or intermittent hypoxia adaptation (7). For example, opening of K_{ATP} in cardiac myocytes offers a protective effect on the heart against ischemia/reperfusion or hypoxia/reoxygenation injury (30). Our previous study has shown that CIHH protects the heart against ischemia/reperfusion injury through the opening of the K_{ATP} channel in rat myocardium (29). Accumulated studies have shown that K_{ATP} channels are also present in vascular smooth muscle (VSM) cells (17). In the vascular system, activation of the K_{ATP} channels can hyperpolarize the membrane of VSM cell, relax VSM and enhance activity of the carotid sinus baroreceptor through stretching the wall of VSM (11). A previous study of our department (22) confirmed that the opening of K_{ATP} channels had a facilitating effect on CSB. In the present study, we found Gli, a non-selective blocker of K_{ATP} channels, could partially eliminate the facilitation of CIHH on the CSB implying that opening of K_{ATP} channels is involved in the facilitation of CIHH on the CSB. Results mentioned above support a hypothesis that the K_{ATP} channel plays an important role in the effect of CIHH not only for the cardiac protection, but also for the facilitation of baroreflex. The precise mechanism of this observation needs further investigation.

It is well known that NO plays an important functional roles in a variety of physiological systems including the vasculature, the nervous and the immune systems. NOS is present in the sensory neurons that dominate the area of carotid sinus implying that NO could modulate baroreceptor activity (19). Overproduction of NO can depress the activity of carotid baroreflex (10), but the effect of basal NO on the CSB is unclear. In the present study, no changes were observed by perfusing the carotid sinus with K-H solution containing *L*-NAME (100 μM) in control animals suggesting that the basal NO has no effect on baroreceptor activities. Available data on the effects of hypoxia on NO and/or NOS are rather controversial (16, 20), which are probably influenced by the use of different intermittent hypoxia protocols, organs and/or cells and different assays. In our experiments, perfusing the carotid sinus with K-H solution containing *L*-NAME had no effect on facilitation of CSB in CIHH rats which suggests that NO does not play any role in the facilitation of CSB offered by CIHH.

The results of this study are not consistent with several recent studies. For example, Fu's results (4) showed that intermittent hypobaric hypoxia had a negative effect on autonomic control of blood

pressure, while our result showed a facilitation of CIHH on CBS. There are two major reasons that may be involved in the discrepancy. Firstly, the experimental objectives were different: young athletes (10 runners and 12 swimmers) were used in Fu's study but anesthetized rats were used in our study. Secondly, or most importantly, the protocols of intermittent hypobaric hypoxia were different. In Fu's study, they used hypobaric hypoxia of 3 h/day, 5 days/week for a total of 20 days (4,000 m in days 1–2, 4,500 m in days 3–4, 5,000 m in days 5–6, and 5,500 m in days 7–20). In our study, we used 5,000 m intermittent hypobaric hypoxia of 6 h/day for 14 and 28 days, respectively. It is known that the effects of CIHH on the cardiovascular function are critically dependent on experiment protocols such as the cycle length of intermittent hypoxia, the number of hypoxic episodes per day, the number of days of exposure and the degree and duration of hypoxic exposure (2).

The limitation of the methodology of this study is the use of anaesthetized animals and that baroreflex may be changed under the anaesthetized condition. Even though many studies on baroreflex used anaesthetized animals (5, 18), it is better to use conscious animals to test baroreflex. Furthermore, the experimental procedures for the activation of the carotid sinus baroreceptor need improving. In the experiment, the CSB was activated based on the program that was designed by our laboratory, lifting ISP from 0 mmHg to 250 mmHg. Indeed, we found that the effective range of the CSB was approximately from 40 mmHg (when the receptor stops firing) to 180 mmHg (when the firing rate reaches a maximum), and there was no significant difference of reflex effect induced by the two ISP protocols. Hence, the latter protocol is more physiological and easy to be understood.

Taken together, chronic intermittent hypobaric hypoxia facilitated CSB through opening K_{ATP} channels in rat carotid sinus area, which may contribute to maintaining the homeostasis of SABP in different pathologic situations such as acute hypoxia.

Acknowledgments

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References

1. Akrouh, A., Halcomb, S.E., Nichols, C.G. and Sala-Rabanal, M. Molecular biology of K_{ATP} channels and implications for health and disease. *IUBMB Life* 61: 971-978, 2009.
2. Béguin, P.C., Joyeux-Faure, M., Godin-Ribuot, D., Lévy, P. and Ribuot, C. Acute intermittent hypoxia improves rat myocardium tolerance to ischemia. *J. Appl. Physiol*. 99: 1064-1069, 2005.
3. Behm, R., Honig, A., Griethe, M., Schmidt, M. and Schneider, P. Sustained suppression of voluntary sodium intake of spontaneously hypertensive rats (SHR) in hypobaric hypoxia. *Biomed. Biochem. Acta* 43: 975-985, 1984.
4. Fu, Q., Townsend, N.E., Shiller, S.M., Martini, E.R., Okazaki, K., Shibata, S., Truijens, M.J., Rodríguez, F.A., Gore, C.J., Stray-Gundersen, J. and Levine, B.D. Intermittent hypobaric hypoxia exposure does not cause sustained alterations in autonomic control of blood pressure in young athletes. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 292: R1977-R1984, 2007.
5. Gu, H., Lin, M., Liu, J., Gozal, D., Scrogin, K.E., Wurster, R., Chapleau, M.W., Ma, X. and Cheng, Z.J. Selective impairment of central mediation of baroreflex in anesthetized young adult Fischer 344 rats after chronic intermittent hypoxia. *Am. J. Physiol. Heart Circ. Physiol*. 293: H2809-H2818, 2007.
6. Katiukhin, V.N., Shliakhto, E.V. and Shuiskaia, G.A. Effect of discontinuous high-altitude barotherapy on the hemodynamics in arterial hypertension. *Kardiologiia* 19: 107-108, 1979 (in Russian).
7. Kolar, F., Nekar, J., Ostadal, B., Maslov, L.N., Stakheev, D.L., Tayurskaya, A.S. and Lishmanov, Y.B. Role of ATP-sensitive K^+-channels in antiarrhythmic and cardioprotective action of adaptation to intermittent hypobaric hypoxia. *Bull. Exp. Biol. Med.* 145: 418-421, 2008.
8. Leuenberger, U.A., Brubaker, D., Quraishi, S., Hogeman, C.S., Imadojemu, V.A. and Gray, K.S. Effects of intermittent hypoxia on sympathetic activity and blood pressure in humans. *Auton. Neurosci.* 121: 87-93, 2005.
9. Li, J., Zhang, H., Zhu, W.Z., Yu, Z., Guo, A., Yang, H.T. and Zhou, Z.N. Preservation of the pHi during ischemia *via* PKC by intermittent hypoxia. *Biochem. Biophys. Res. Commun*. 356: 329-333, 2007.
10. Meyrelless, S.S., Sharma, R.V., Mao, H.Z. Abboud, F.M. and Chapleau, M.W. Modulation of baroreceptor activity by gene transfer of nitric oxide synthase to carotid sinus adventitia. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 284: 1190-1198, 2003.
11. Morris, C.E. Mechanosensitive ion channels. *J. Membr. Biol*. 113: 93-107, 1990.
12. Pfeffer, J.M., Pfeffer, M.A. and Frohlich, E.D. Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. *J. Lab. Clin. Med*. 78: 957-962, 1971.
13. Powell, F.L. and Garcia, N. Physiological effects of intermittent hypoxia. *High Alt. Med. Biol*. 1: 125-136, 2000.
14. Quayle, J.M., Bonev, A.D., Brayden, J.E. and Nelson, M.T. Pharmacology of ATP-sensitive K^+ currents in smooth muscle cells from rabbit mesenteric artery. *Am. J. Physiol*. 269: C1112-C1118, 1995.
15. Quayle, J.M. and Standen, N.B. K_{ATP} channels in vascular smooth muscle. *Cardiovasc. Res*. 28: 797-804, 1994.
16. Rodway, G.W., Sethi, J.M., Hoffman, L.A., Conley, Y.P., Choi, A.M., Sereika, S.M., Zullo, T.G., Ryter, S.W. and Sanders, M.H. Hemodynamic and molecular response to intermittent hypoxia (IH) versus continuous hypoxia (CH) in normal humans. *Transl. Res*. 149: 76-84, 2007.
17. Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y. and Nelson, M.T. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* 245: 177-180, 1989.
18. Vitela, M., Herrera-Rosales, M., Haywood, J.R. and Mifflin, S.W. Baroreflex regulation of renal sympathetic nerve activity and heart rate in renal wrap hypertensive rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 288: R856-R862, 2005.
19. Wang, Z.Z., Bredt, D.S., Fidone, S.J. and Stensaas, L.J. Neurons synthesizing nitric oxide innervate the mammalian carotid body. *J. Comp. Neurol*. 336: 419-432, 1993.
20. Xiao, D., Bird, I.M., Magness, R.R., Longo, L.D. and Zhang,

L. Upregulation of eNOS in pregnant ovine uterine arteries by chronic hypoxia. *Am. J. Physiol. Heart Circ. Physiol*. 280: H812-H820, 2001.

21. Xiao, L., Wu, Y.M., Zhang, H., Liu, Y.X. and He, R.R. Hydrogen sulfide facilitates carotid sinus baroreflex in anesthetized rats. *Acta Pharmacol. Sin*. 27: 294-298, 2006.
22. Yin, T., Chen, S. and He, R.R. K_{ATP} channel opener facilitates carotid sinus baroreflex in anaesthetized rats. *Acta Physiol. Sin*. 52: 170-174, 2000.
23. Yi, X.L., Fan, Z.Z. and Ho, S.Y. An automatic system controlled by computer for carotid sinus perfusion. *Chin. J. Apply. Physiol*. 9: 156-159, 1993.
24. Zhang, Y., Zhong, N., Gia, J.Y. and Zhou, Z.N. Effects of chronic intermittent hypoxia on the hemodynamics of systemic circulation in rats. *Jpn. J. Physiol*. 54: 171-174, 2004.
25. Zhang, Y., Zhong, N. and Zhou, Z.N. Effects of intermittent hypoxia on action potential and contraction in non-ischemic and ischemic rat papillary muscle. *Life Sci*. 67: 2465-2471, 2000.
26. Zhang, Y., Zhong, N., Zhu, H.F. and Zhou, Z.N. Antiarrhythmic and antioxidative effects of intermittent hypoxia exposure on rat myocardium. *Acta Physiol. Sin*. 52: 89-92, 2000.
27. Zhong, N., Zhang, Y., Fang, Q.Z. and Zhou, Z.N. Intermittent hypoxia exposure-induced heat-shock protein 70 expressions increases resistance of rat heart to ischemic injury. *Acta Pharmacol. Sin*. 21: 467-472, 2000.
28. Zhong, N., Zhang, Y., Zhu, H.F., Wang, J.C., Fang, Q.Z. and Zhou, Z.N. Myocardial capillary angiogenesis and coronary flow in ischemia tolerance rat by adaptation to intermittent high altitude hypoxia. *Acta Pharmacol. Sin*. 23: 305-310, 2002.
29. Zhu, H.F., Dong, J.W., Zhu, W.Z., Ding, H.L. and Zhou, Z.N. ATP-dependent potassium channels involved in the cardiac protection induced by intermittent hypoxia against ischemia/reperfusion injury. *Life Sci*. 73: 1275-1287, 2003.
30. Zingman, L.V., Alekseev, A.E., Hodgson-Zingman, D.M. and Terzic, A. ATP-sensitive potassium channels: metabolic sensing and cardioprotection. *J. Appl. Physiol*. 103: 1888-1893, 2007.