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Original Article

Amplified cardiorespiratory activity by hypoxia in conscious spontaneously hypertensive rats

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Abstract: Activation of peripheral respiratory chemoreceptors provokes respiratory and cardiovascular reflexes, providing a novel understanding of pathogenic mechanism of hypertension. Here we hypothesize that activation of peripheral respiratory chemoreceptors by hypoxia causes enhanced cardiorespiratory activity in conscious spontaneously hypertensive rats (SHRs). Using whole body plethysmography in combination with radio telemetry, pulmonary ventilation, arterial blood pressure and heart rate were examined in SHRs and Wistar-Kyoto (WKY) rats. We found that exposure to hypoxia induced greater increases in tidal volume and minute ventilation volume in SHRs compared to WKY rats. In addition, hypoxia caused a robust increase in arterial blood pressure and heart rate in SHRs relative to WKY counterparts. After carotid body denervation, the hypoxic ventilatory response was significantly decreased in both SHRs and WKY rats, but without significant difference between the two strains; moreover, the differences of arterial blood pressure and heart rate changes during hypoxic exposure were statistically insignificant between SHRs and WKY rats. It is concluded that hypoxia remarkably potentiates cardiorespiratory activity in the SHRs, suggesting an enhanced sensitivity of carotid bodies to hypoxia.

Key words: carotid body; ventilation; hypertension; hypoxia

低氧增强清醒自发性高血压大鼠心血管-呼吸活动

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摘 要：激活外周化学感受器可以同时引起呼吸和心血管反射，此机制可能参与高血压形成过程中的交感神经过度激活。因此我们推测激活外周呼吸化学感受器可以显著增强高血压大鼠的心肺活动。本研究通过联合应用全身无创全体积描记术和无线生物信号遥测技术，观察急性低氧刺激对清醒自发性高血压大鼠(spontaneously hypertensive rat, SHR)和血压正常的对照Wistar-Kyoto (WKY)大鼠的肺通气、动脉血压和心率的影响。结果表明，急性低氧刺激引起SHR潮气量和每分通气量明显高于WKY大鼠，并且急性低氧引起SHR血压和心率的增加幅度更明显。切断支配大鼠颈动脉体的双侧窦神经后，SHR和WKY大鼠急性低氧通气反应均降低，并且两组间比较没有显著性差异。同时，在切断双侧窦神经后，急性低氧引起的两组动物的血压和心率变化均无显著性差异。本研究表明，急性低氧刺激显著增强SHR的心血管和呼吸效应，这可能与其颈动脉体外周呼吸化学感受器对低氧的敏感性增高有关。

关键词：颈动脉体；肺通气；高血压；低氧
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Essential hypertension is a complex, multifactorial and widespread disease. It is also a high risk factor for cardiovascular diseases such as stroke, myocardial infarction, and heart failure. Enhanced sympathetic nerve activity (SNA) has been linked to the pathogenesis of hypertension in humans and animal models [1, 2].

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However, what promotes heightened SNA has not been fully understood. More recently, attention is gained to respiratory modulation of SNA and arterial blood pressure in spontaneously hypertensive rats (SHRs), and in particular, dysfunctions of peripheral and central respiratory chemoreceptors may contribute to the development of hypertension [3–5]. Therefore, it has been proposed that augmented respiratory-sympathetic coupling contributes to the development of hypertension, which is a demonstrable mechanism underlying neurogenic hypertension [6].

The carotid body (CB) chemoreceptors, located near the fork of the carotid artery, are activated shortly after exposure to hypoxia and then send information through carotid sinus nerves to the nucleus tractus solitarii and higher integrative centers, with the predominant regulation of cardiorespiratory activity. Sympathetic overactivity may result directly or indirectly from hypersensitivity of CB chemoreceptors in neurogenic hypertension, obstructive sleep apnea and metabolic disorders [7–9]. Hypoxic stimulation of CB chemoreceptors altered the pattern of respiratory phase-modulated SNA in anesthetized SHRs rather than normotensive Wistar-Kyoto (WKY) rats [10]. Moreover, hypersensitivity of CB chemoreceptors to hypoxia already occurred in anesthetized young SHRs prior to hypertension onset [4]. Furthermore, the CB's denervation preceding hypertension onset in conscious SHRs reduced the sympathetic overactivity and hence, lowered arterial blood pressure, in favor of a therapeutic role of CB chemoreceptors in the development of neurogenic hypertension [3, 8]. However, it remains unresolved whether hypoxic activation of CB chemoreceptors differentially affects the cardiorespiratory pattern in conscious SHRs. Given hypersensitivity of peripheral chemoreceptors, the cardiorespiratory responses to hypoxia most likely contributes to the development of hypertension. Hence, this issue awaits to be addressed.

By employing whole body plethysmography (WBP) in combination with radio telemetry, we got access to examining simultaneously the ventilatory response and cardiovascular activity in conscious rats, and tested the hypothesis that the CB's hypersensitivity contributes to amplified cardiorespiratory activity in the SHRs.

1 MATERIALS AND METHODS

1.1 *Animals*

All experiments were performed in accordance with ethical guidelines of the Animal Protection Association and were approved by Animal Care and Ethical Committee of Hebei Medical University. Male SHRs and age-matched normotensive WKY rats were used in the study (12–15 weeks old, body weight 250–300 g). Animals, synchronized for a 12:12 h light-dark cycle (lights on at 8 am, lights off at 8 pm), were housed individually and allowed to freely move in standard plastic cages in a climate-controlled room (22 °C ± 1 °C). Food and water were provided *ad libitum*. The whole experimental procedure was presented in Fig. 1. When the animal experiments were completed, intraperitoneal injection of an overdose of sodium pentobarbital (> 200 mg/kg) was carried out for euthanasia.

1.2 *Arterial pressure measurements and manipulation*

Arterial blood pressure was measured with radio telemetry system in rats. General anesthesia was induced with 4% halothane in 100% O_2 and maintained by reducing the inspired halothane concentration to 1.5%–

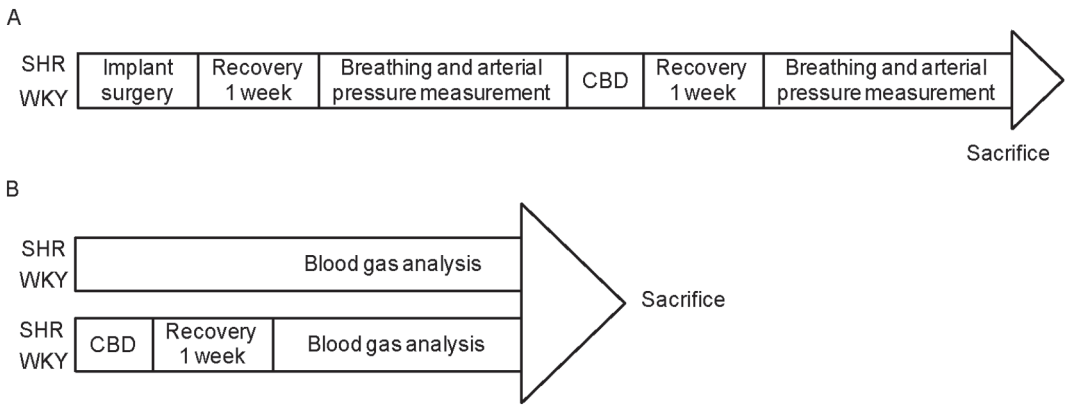

Fig. 1. Experiment design. *A*: Cardiopulmonary function determination process. *B*: Blood gas analysis experiment. CBD: carotid body denervation.

1.8%. The depth of anesthesia was assessed by an absence of the corneal and hindpaw withdrawal reflex. Body temperature of all rats was maintained at 37 °C using a temperature-controlled heating pad. Under aseptic conditions, the polyethylene tube of the implant was inserted into the left femoral artery until its tip reached the abdominal aorta. The radio telemeters (Data Sciences Inc., St Paul, Minnesota, USA) was placed in the abdominal subcutaneous (parallel to the body axis) and attached to the muscle wall. After surgery, all animals received an intramuscular injection of penicillin G (24 000 IU), streptomycin (10 mg) and given the analgesic Ketoprofen for two days (s.c., 4 mg/kg per day). Rats were individually housed and given 7 days for recovery. Arterial blood pressure was recorded and analyzed by the software Dataquest ART Acquisition (version 4.33, Data Sciences Inc., USA). Rats whose transmitters failed were excluded from analysis. Cardiovascular parameters measured by radio telemetry included systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP). Heart rate (HR) was derived from blood pressure waveforms.

1.3 *Measurement of breathing parameters*

Breathing parameters were measured in conscious, freely moving rats by WBP (EMKA Technologies, France) as described previously [11, 12]. The measurement was conducted following one week recovery from the radio telemeter implant surgery. Briefly, rats were placed in the chamber ahead of the testing protocol for at least 2 h (for acclimation). Rats were exposed to normoxia (21% O_2, balance N_2) for 10 min, followed by hypoxia (10% O_2, balance N_2) for up to ~5 min using a gas mixture device (1.5 L/min, GSM-3, CWE, USA). Ventilatory flow signals were recorded, amplified, digitized and analyzed using IOX 2.7 (EMKA Technologies) to determine respiratory parameters over sequential 20 s epochs during periods of behavioral quiescence. Minute ventilation volume [MV, μL/(min·g)] was calculated as the product of the respiratory frequency (RF, breaths/min) and tidal volume (TV, μL/g), normalized to rat body weight (g). The WBP in combination with radio telemetry allowed to simultaneously measure respiratory and cardiovascular parameters in conscious rats so that the cardiorespiratory activity was accessed under physiological conditions.

1.4 *Carotid body denervation (CBD)*

To further confirm the CB-mediated effect, cardiorespiratory parameters were also measured in rats with CBD. The carotid sinus nerve branches were sectioned as previously described [11]. Shortly, general anesthesia was carried out and assessed as depicted hereinabove. After exposure of carotid artery branches, the carotid sinus nerves were removed completely from the cranial pole of the CB until reaching the branch to the glossopharyngeal nerve. The wound was carefully sutured and disinfected with 10% polividone iodine. All animals were received an intraperitoneal injection of penicillin G (24 000 IU) and streptomycin (10 mg) after the surgery. Conscious chemodenervated rats were exposed to hypoxia 5–7 days after recovery.

1.5 *Arterial blood gas analysis*

The protocol has been described previously [11]. In brief, arterial blood gas was measured using an OPTI-CCA blood gas analyzer (OPTI Medical Systems, USA) at a steady state in halothane-anesthetized, paralyzed rats. After general anesthesia, arterial blood (200 μL per sample) was drawn from the femoral artery in rats. The arterial oxygen saturation, partial pressure of arterial O_2 (P_aO_2), partial pressure of CO_2 (P_aCO_2) and blood pH were measured.

1.6 *Statistical analysis*

Statistical analysis was performed with Prism version 7 (GraphPad Software Inc., La Jolla, CA, USA). Values are presented as mean ± SEM unless indicated otherwise. Data were compared by Student's *t* test or two-way ANOVA followed by Tukey's tests. Differences within or between groups with $P < 0.05$ were considered significant.

2 RESULTS

2.1 Baseline breathing parameters measured by WBP

Baseline breathing parameters were measured during normoxia (21% O_2) in conscious SHRs and WKY rats ($n = 10$ for each group) when they were quietly resting (Fig. 2). As a result, basal RF and MV were insignificantly different between two strains of rats (Fig. 3*A&C*), with the exception that the SHRs displayed a larger TV in relative to the WKY counterparts [(5.3 ± 0.3) μL/g *vs* (4.1 ± 0.2) μL/g, $P < 0.01$, Fig. 3*B*], suggesting a slower and deeper ventilation pattern in the SHRs.

2.2 Enhanced ventilatory response to hypoxia in SHR

Acute exposure to 10% O_2 (relative to room air) con-

siderably increased both RF ($P < 0.05$, Fig. 3*A*) and MV ($P < 0.01$, Fig. 3*C*), rather than TV ($P > 0.05$, Fig. 3*B*), in both WKY rats and SHRs ($n = 10$ for each group) with carotid body innervation (CBI). And the increment of MV was larger in SHRs compared to WKY rats ($P < 0.05$, Fig. 3*G*). During exposure to hypoxia, the SHRs had larger TV [(5.8 ± 0.3) μL/g *vs* (4.6 ± 0.2) μL/g, $P < 0.01$, Fig. 3*B*] and MV [(627 ± 34) *vs* (480 ± 24) μL/(min·g), $P < 0.01$, Fig. 3*C*] when compared to the WKY rats, whereas both strains had

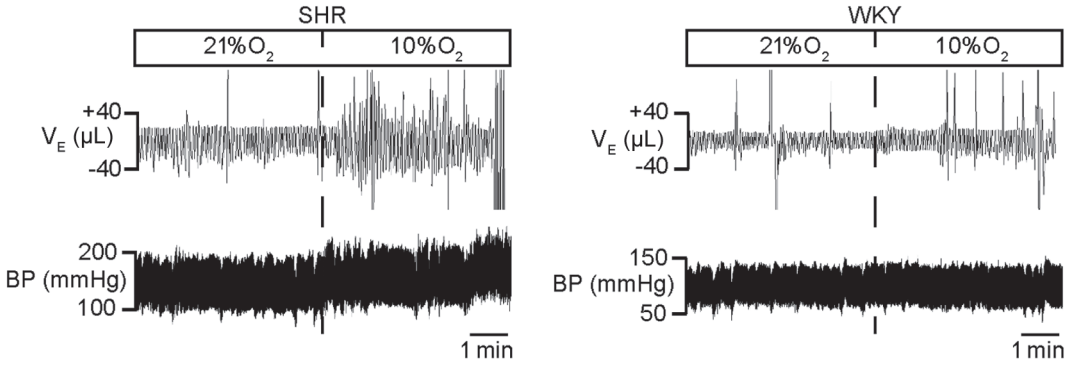

Fig. 2. Representative recording of cardiopulmonary responses to hypoxic exposure in conscious SHRs and WKY rats. Typical traces showing the effect of hypoxic exposure on cardiopulmonary activity in conscious SHRs and WKY rats. V_E: minute ventilation volume; BP: blood pressure.

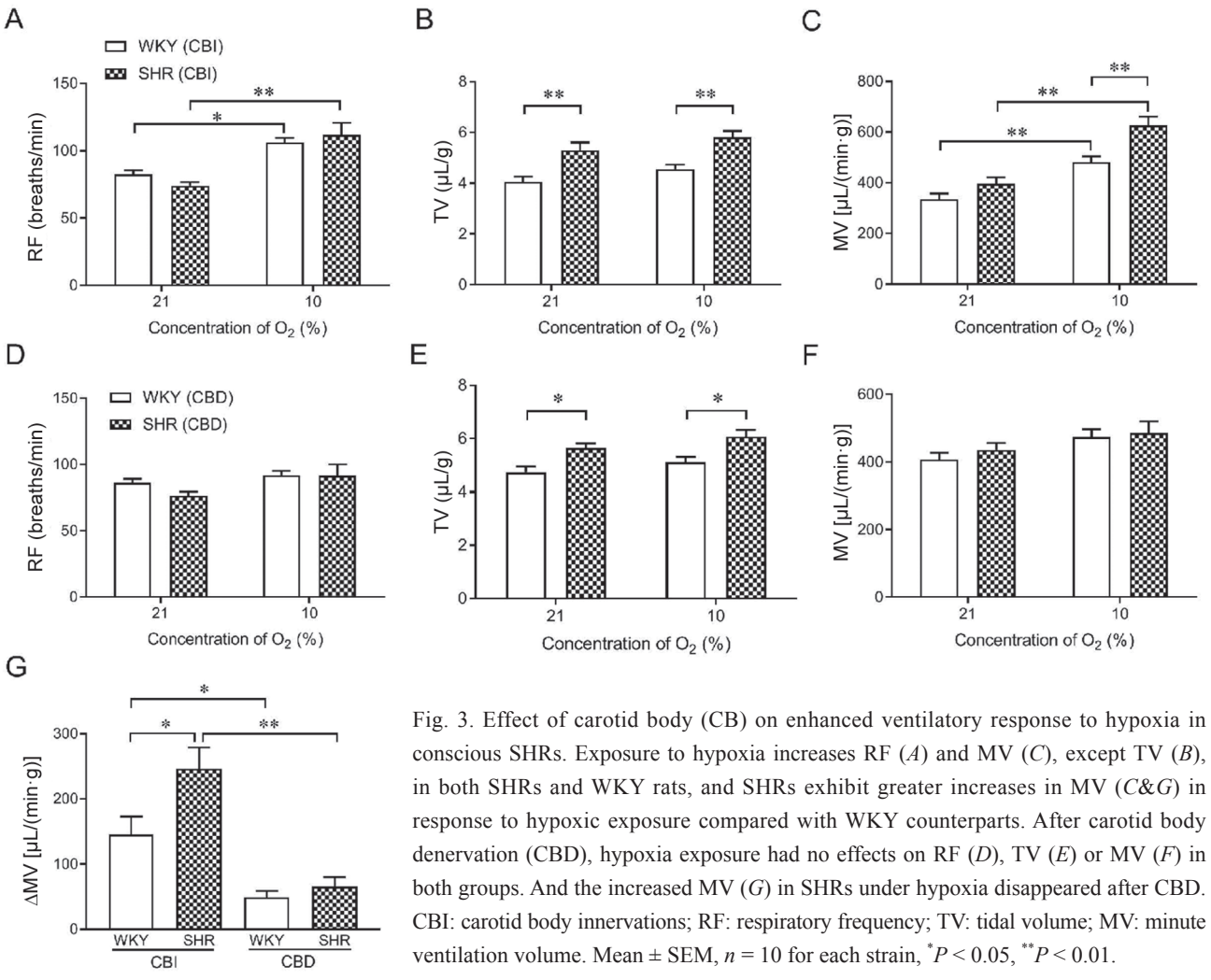

Fig. 3. Effect of carotid body (CB) on enhanced ventilatory response to hypoxia in conscious SHRs. Exposure to hypoxia increases RF (*A*) and MV (*C*), except TV (*B*), in both SHRs and WKY rats, and SHRs exhibit greater increases in MV (*C*&*G*) in response to hypoxic exposure compared with WKY counterparts. After carotid body denervation (CBD), hypoxia exposure had no effects on RF (*D*), TV (*E*) or MV (*F*) in both groups. And the increased MV (*G*) in SHRs under hypoxia disappeared after CBD. CBI: carotid body innervations; RF: respiratory frequency; TV: tidal volume; MV: minute ventilation volume. Mean ± SEM, $n = 10$ for each strain, $^{*}P < 0.05$, $^{**}P < 0.01$.

the similar degree of RF ($P > 0.05$, Fig. 3*A*).

After CBD, no considerable difference in RF or MV was observed between SHRs and WKY rats when exposure to 10% O_2 in inspired air ($P > 0.05$, Fig. 3*D*&*F*). After CBD, SHRs had larger TV under both normoxia and hypoxia compared to WKY rats ($P < 0.05$, Fig. 3*E*). Furthermore, hypoxia-elicited increment of MV between two strains was without significant difference after sectioning carotid sinus nerves ($P > 0.05$, Fig. 3*G*), in support of the contribution of CBs. Thus, the enhanced ventilatory response to hypoxia in SHRs was mainly due to activation of CBs.

2.3 Effect of hypoxia on cardiovascular activity

The radio telemetry in combination with WBP allows to measure simultaneously respiratory and cardiovascular parameters (Fig. 2). In addition to affecting breathing parameters as described hereinabove, acute exposure to 10% O_2 also caused slight increasing in SBP from (187 ± 5) to (195 ± 5) mmHg, DBP from (132 ± 7) to (139 ± 5) mmHg and the according MAP from (160 ± 5) to (167 ± 5) mmHg in SHRs ($n = 7$, Fig. 4*A–C*), despite with no statistical significance ($P > 0.05$). While the blood pressure was almost unchanged in WKY rats ($n = 7$, Fig. 4*A–C*). Likewise, the increment in MAP (difference in MAP between hypoxia and normoxia) in SHRs was larger than that in WKY rats [(9 ± 3) *vs* (1 ± 2) mmHg, $P < 0.05$, Fig. 4*D*]. Meanwhile, under hypoxia the HR in SHRs was faster than that in WKY rats [(335 ± 13) *vs* (296 ± 6) beats/min, $P < 0.05$, Fig. 4*E*]. Of importance, the hypoxia-induced HR increment (difference

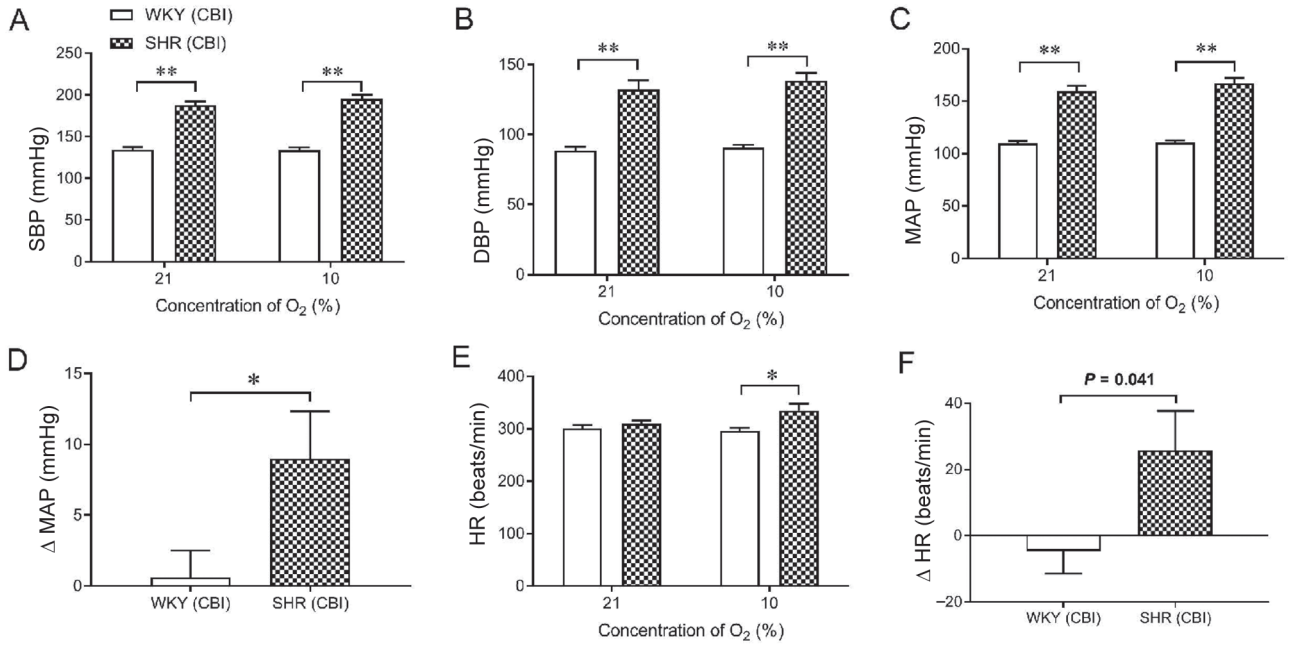

Fig. 4. Hypoxia reinforced cardiovascular activity in conscious SHRs. Hypoxic stimulation causes slight increases in SBP (*A*), DBP (*B*) and MAP (*C*) in SHR rather than WKY rats, although with no statistical significance. The HR was faster in SHRs compared to WKY rats under acute hypoxia (*E*). In addition, after exposure to hypoxia, the increments of MAP (*D*) and HR (*F*) were larger in SHRs than WKY counterparts. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; HR: heart rate; CBI: carotid body innervations. Mean ± SEM, $n = 7$ for each strain, $^{*}P < 0.05$, $^{**}P < 0.01$.

Table 1. Baseline cardiovascular parameters

	CBI		CBD	
	SHR	WKY	SHR	WKY
Body weight (g)	271 ± 4	278 ± 4	264 ± 3	269 ± 4
SBP (mmHg)	187 ± 5**	135 ± 3	188 ± 4**	135 ± 2
DBP (mmHg)	132 ± 7**	89 ± 3	131 ± 3**	94 ± 2
HR (beats/min)	310 ± 6	300 ± 7	312 ± 6	297 ± 7

Values were mean ± SEM, $n = 7$ for each strain in carotid body innervation (CBI) rats, $n = 20$ for each strain in carotid body denervation (CBD) rats. $^{**}P < 0.01$ *vs* corresponding WKY rats.

in HR between hypoxia and normoxia) in the SHRs was larger than that in the WKY rats [(26 ± 12) *vs* (−5 ± 7) beats/min, $P < 0.05$, Fig. 4*F*], suggesting an enhanced sympathetic outputs by hypoxia in the SHRs.

After CBD, actually there was no obvious difference in blood pressure or HR in both SHRs and WKY rats when compared to their CBI counterparts ($P > 0.05$, Table 1). After sectioning carotid sinus nerves, the changes caused by hypoxia exposure in MAP and HR were statistically insignificant between the two strains (Fig. 5). Therefore, the CB mediated the stimulatory effect of hypoxia on sympathetic outputs in the SHRs.

2.4 Blood gas analysis

To rule out the possibility that different levels of blood O_2, CO_2 and pH values in two strains ($n = 5$ for each) may contribute to differential cardiorespiratory responses, experiments were performed to measure blood gas. Acute hypoxic exposure decreased both P_aO_2 and arterial oxygen saturation in CBI and CBD SHRs and WKY rats ($P < 0.05$–0.01) compared with those measured during normoxia, while no difference was found between strains ($P > 0.05$, Fig. 6*A*&*B*).

As for the P_aCO_2 and blood pH values, no difference was found between SHRs and WKY rats with or without CBI in normoxia ($P > 0.05$, Fig. 6*C*&*D*). Hypoxic exposure had no marked effect on the level of P_aCO_2 between strains. Acute hypoxia exposure significantly increased the pH values in CBI SHRs as well as CBI WKY rats ($P < 0.05$–0.01, Fig. 6*D*), while no difference was found between the two strains ($P > 0.05$). Altogether, the normal resting blood gas parameters observed in two strains of rats did not contribute to differential cardiorespiratory activity.

3 DISCUSSION

In the present study, we demonstrate that acute exposure to hypoxia produced enhanced ventilatory responses in the SHRs, as well as robust increases in arterial blood pressure and HR. Such cardiorespiratory responses to hypoxia were abolished when submitted to CBD. This suggests that the hypersensitivity of CBs to hypoxia contributes to enhanced ventilatory responses and sympathetic outputs in the SHRs.

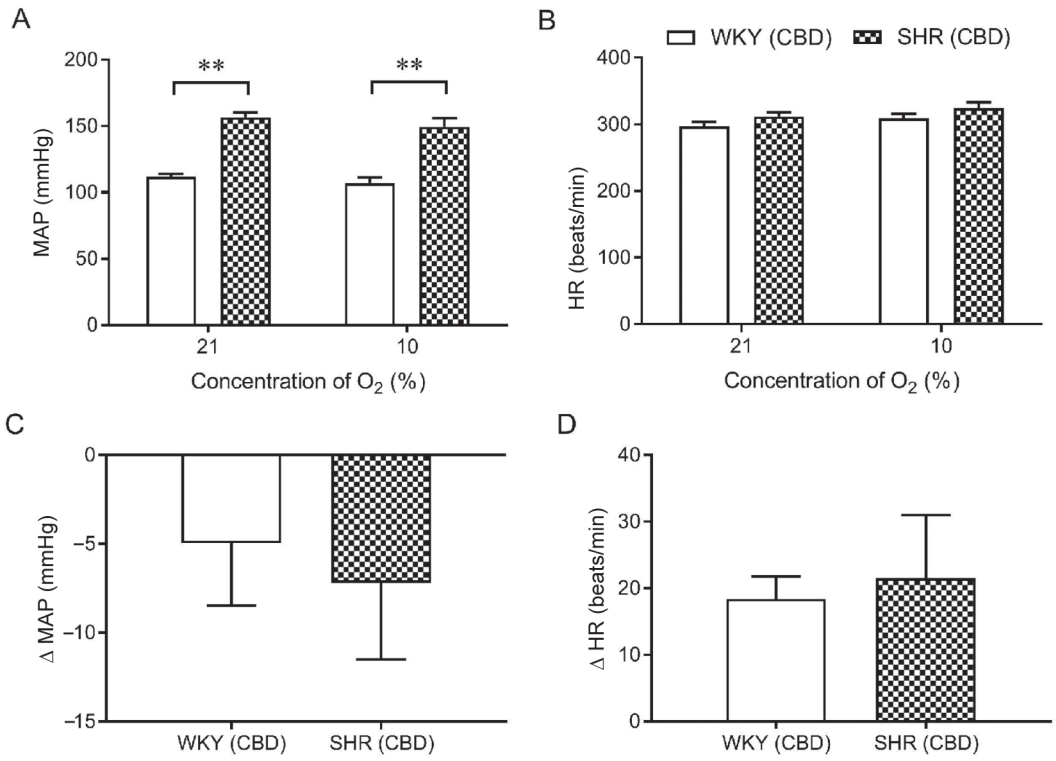

Fig. 5. Effect of carotid body (CB) on cardiovascular activity in SHRs and WKY rats under hypoxia. After carotid body denervation (CBD), MAP (*A*) was slightly decreased while HR (*B*) was increased by hypoxic stimulation in both SHR and WKY rats, although with no statistical significance. There was no difference in the magnitude of changes in MAP (*C*) or HR (*D*) between the two rat strains. Mean ± SEM, $n = 20$ for each strain, $^{**}P < 0.01$.

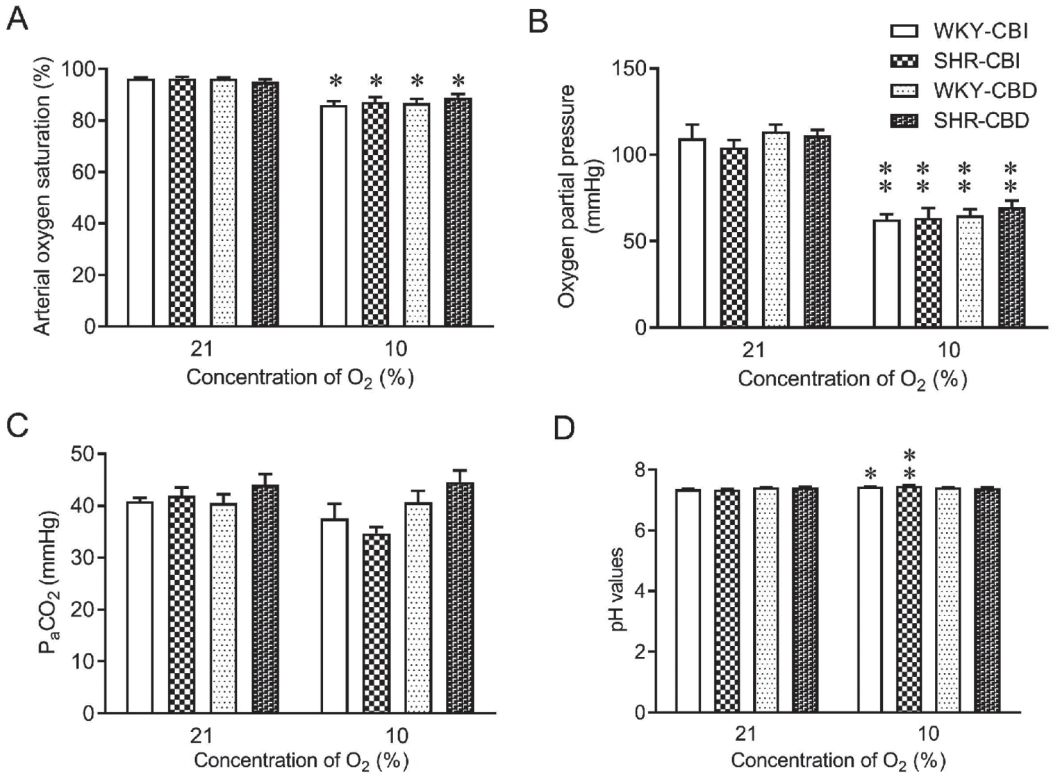

Fig. 6. Blood gas analysis during acute hypoxic exposure. When exposure to hypoxia (10% O_2), arterial oxygen saturation (*A*) and oxygen partial pressure (P_aO_2, *B*) significantly decreased compared to normoxia (21% O_2) in both group rats, while no difference was found in carbon dioxide partial pressure (P_aCO_2, *C*). Acute hypoxia significantly increased pH of blood in carotid body innervation (CBI) SHRs and WKY rats, while no difference was found in carotid body denervation (CBD) rats (*D*). Mean ± SEM, $n = 5$ for each strain, $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* corresponding normoxic status.

The peripheral respiratory chemoreceptors, consisting of CBs and aortic body, provide essential afferent inputs required for regulation of breathing. The CB is the major arterial oxygen sensor and sensitive to hypoxia and hypercapnia through type I cells, hence eliciting adaptive ventilation. The sensitivity of peripheral chemoreceptors has been documented to be enhanced in both patients with neurogenic hypertension and SHRs [13–15]. We have shown herein that under normoxic condition, SHRs display a larger TV compared with WKY rats. Further, hypoxia elicits stronger increases in MV in SHRs. It is hence reasonably speculated that hypersensitivity of CB chemoreceptors in SHRs contributes to enhanced ventilatory responses to hypoxia. After CBD, the SHRs manifest similar ventilatory response to hypoxia compared to the WKY counterparts, suggesting that the enhanced hypoxic ventilatory responses are CB-mediated in the SHRs. However, we cannot rule out that such enhanced ventilatory response in the SHRs is exclusively attributable to hypersensitivity of CB chemoreceptors because other peripheral mechanisms may operate (e.g. airway resistance, neuromuscular junction).

In addition to respiratory actions, the hypersensitivity of CB chemoreceptors has also been implicated in contributing to heightened sympathetic traffic in SHRs [4, 16]. Based on data obtained herein, the SHRs exhibited a larger increase in HR in response to hypoxia in relative to the WKY counterparts, most likely ascribing to heightened cardiac sympathetic activity, but we can't rule out the humoral contribution. Then we sectioned the bilateral carotid sinus nerves in both rat strains, which were confirmed by reduced hypoxic ventilatory response. No significant difference in MV during hypoxia was found between two strains with CBD. According to recent reports, carotid sinus nerve denervation results in an attenuation in the blood pressure in SHRs, indicating a possibility that augmented CB chemoreceptor drive contributes to sympathetic outflows [8, 16]. Consistent with these findings, the present data suggest that the hypersensitivity of CB chemoreceptors in SHRs may elicit more excitatory drive to sympathetic

outflow that subsequently contributes to hypertension.

Respiratory and cardiovascular functions are precisely controlled by complex neural networks [17]. Such autonomic reflex adjustments are observed under conditions of hypoxia [18]. Excitatory inputs from the peripheral chemoreceptors activate brainstem neural circuits, boost the respiratory reflexes [19], and meanwhile produce an reinforced sympathetic outflow to elevate arterial pressure [20]. Note that the issue is needed to be addressed with regard to which factors contribute to the hypersensitivity of CB chemoreceptors in the SHRs, for instance, genetics, inflammation, oxidative stress and so on. The hypersensitivity of CB in the SHRs may result from increased expression of acid-sensing sodium channels 3 and 2-pore domain acid-sensitive K^+ channels [4]. The enhanced expression of neurotrophic factors and the corresponding receptors in the hypertensive CB contribute to its hypersensitivity [14]. Heightened sympathetic drive to the arterioles of the CB *per se* may also contribute to its hyperactivity because of hypoperfusion (via vasoconstriction and vascular remodeling) [21]. Additionally, the size of hypertensive CB in the SHRs is much larger than that in WKY rats [22, 23], suggesting that morphological alterations of CB may be a causative factor.

In summary, the hypersensitivity of CB chemoreceptors to hypoxia elicits augmented respiratory-sympathetic activity in the SHRs, gaining a better understanding of the role of respiratory-sympathetic coupling in the pathogenesis of hypertension.

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