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Carotid body and cardiorespiratory alterations in intermittent hypoxia: the oxidative link

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Running head: Intermittent hypoxia and carotid body

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ABSTRACT

Intermittent hypoxia, a feature of obstructive sleep apnea, potentiates ventilatory hypoxic responses, alters heart rate variability and produces hypertension, partially attributed to an enhance carotid body responsiveness to hypoxia. Since oxidative stress is a potential mediator of both chemosensory and cardiorespiratory alterations, we hypothesized that an antioxidant treatment may prevent these alterations.

Accordingly, we studied the effects of ascorbic acid (1.25 g/l drinking water) on plasma lipid peroxidation, nitrotyrosine and iNOS immunoreactivity in the carotid body, ventilatory and carotid chemosensory responses to acute hypoxia, heart rate variability and arterial blood pressure in male Sprague-Dawley rats exposed to 5% O₂; 12 episodes h⁻¹; 8 h day⁻¹ or sham condition for 21 days.

Intermittent hypoxia increased plasma lipid peroxidation, nitrotyrosine and iNOS expression in the carotid body, enhanced carotid chemosensory and ventilatory hypoxic responses, modified heart rate variability and produced hypertension. Ascorbic acid prevented the increased plasma lipid peroxidation and nitrotyrosine formation within the carotid body, the enhanced carotid chemosensory and ventilatory responses to hypoxia, as well as heart rate variability alterations and hypertension.

Present results support an essential role for oxidative stress in the generation of carotid body chemosensory potentiation and systemic cardiorespiratory alterations induced by intermittent hypoxia.

Keywords: Intermittent hypoxia, ascorbic acid, sleep apnea, carotid body.

INTRODUCTION
Obstructive sleep apnea (OSA), a highly prevalent sleep-breathing disorder is recognized as an independent risk factor for hypertension [1,2]. OSA, characterized by recurrent episodes of partial or complete upper airway obstruction during sleep, produces chronic intermittent hypoxia (CIH), which is considered the main factor for hypertension [2,3]. Sympathetic activation, oxidative stress and inflammation have been proposed as potential mechanisms for the OSA-induced hypertension [3-5]. However, conclusive evidence from studies in OSA patients has been difficult to establish because of concomitant co-morbidities [3]. Thus animal CIH models, simulating hypoxic-reoxygenation cycles are used to study OSA pathological mechanisms. Similarly to what is observed in OSA patients, CIH-exposed animals show enhanced sympathetic discharges in response to hypoxia, alterations of heart rate variability (HRV) and develop systemic hypertension [6,7].

It has been proposed that a major contributing mechanism to the CIH-induced sympathetic activation is the potentiation of the carotid body (CB) chemoreflex response to hypoxia [5,7-11]. Indeed, recently diagnosed OSA patients show enhanced ventilatory and cardiovascular hypoxic responses attributed to a CB chemosensory potentiation [9,10]. Moreover, studies performed in animal models provided evidence that CIH enhances CB chemosensory and ventilatory responses to hypoxia and produces long-term facilitation of respiratory motor activity [12,13]. The mechanisms underlying the CIH-induced CB chemosensory potentiation are not well known. The enhanced CB hypoxic reactivity has been attributed to increased levels of endothelin-1 [14] and free radicals in the CB [12,15], but nitric oxide (NO) and pro-inflammatory molecules may also be involved [8]. Since reactive oxygen (ROS) and nitrogen species (RNS) are potential mediators of cardiovascular alterations in OSA patients [3-5] and CIH-exposed
animals [15-17], we hypothesized that an antioxidant treatment may prevent both CB and cardiorespiratory altered functions. Thus, we study the effects of ascorbic acid on the potentiation of CB chemosensory and ventilatory hypoxic responses, HRV alterations and the hypertension induced by CIH in rats. Malondialdehyde assay was used as a systemic oxidative stress marker, while 3-nitrotyrosine (3-NT) and inducible nitric oxide synthase (iNOS) immunoreactivity as oxidative stress markers in the CB. This study was performed in anesthetized rats, a necessary condition to record CB chemosensory neural activity.
METHODS

Animals and intermittent hypoxic exposure
Experiments were performed on male Sprague-Dawley rats (200 g), fed with standard diet ad libitum and kept on a 12-hour light/dark schedule (8:00 am-8:00 pm). The protocol was approved by the Bioethical Committee of the Biological Sciences Faculty, P. Universidad Católica de Chile. Unrestrained, freely moving rats housed in individual chambers (12 cm x 35 cm, 2.2l) were exposed to 5% inspired O₂ for 20s followed by room air for 280s; 12 episodes·h⁻¹; 8 h·day⁻¹ during 21 days. The hypoxic pattern was applied from 8:00 am to 4:00 pm. A computerized system based on solenoid valves controls the alternating cycles of N₂ and room air [7]. In the Sham condition, the N₂ gas was replaced by means of flushing equal flow of compress air into chambers. Room temperature was kept at 23-25°C. Ascorbic acid (1.25 g/l) was administered through the drinking tap water from the first day of CIH exposure. The water solution was freshly prepared every day, and preserved in dark containers to avoid oxidation. Rats were randomly assigned into experimental groups. Ascorbic acid (AA) was administered to rats exposed to CIH (CIH-AA) or sham condition (Sham-AA). Fluid intake was no significantly different between all groups (~10 ml/day).

Physiological Recordings
Recordings were performed ~16 hrs after the end of the last hypoxic cycle. Rats were anesthetized with sodium pentobarbitone (40 mg kg⁻¹ i.p.) followed by additional doses when necessary to maintain a level of surgical anesthesia, placed in supine position and the rectal temperature was maintained at 38.0±0.5°C with a regulated heating pad. The
trachea was cannulated for airflow recording, and connected to a pneumotachograph to measure tidal volume ($V_T$), respiratory frequency ($Fr$), and minute inspiratory volume ($V_i$). One carotid artery was cannulated with a P50-polyethylene tube, filled with 50 IU/ml of heparin solution for measuring arterial blood pressure (BP) with a transducer (Statham P23, USA). However, in experiments designed to record CB chemosensory discharges and ventilatory responses, the carotid artery was not cannulated to avoid any interference with the blood supply of the CB region. Heart rate ($F_H$) was obtained from the ECG signal recorded using the II lead, and the mean arterial blood pressure (MABP) from the BP signal. Signals were acquired with an analog-digital system PowerLAB 8SP, calibrated and analyzed with the Chart 6.1-Pro software (ADInstruments, Australia). To assess the contribution of the peripheral chemoreceptors on the CIH-induced changes in the ventilatory reflex, we measured ventilatory responses elicited by isocapnic levels of PO$_2$ (5 to 670 mmHg), maintained until the response was in a semi steady state (~10-20 s).

**Heart rate variability**

The ECG was recorded prior to any maneuver for 10 min at 2 kHz. After the acquisition, the signal was explored for detection of ectopic QRS complexes and R-R intervals were measured. The heart rate variability (HRV) was analyzed with the HRV module of the Chart 6.1-Pro software. The power spectrum of R-R interval data was obtained using a Fast Fourier Transform algorithm after application of the Hann window. The spectrum of R-R intervals was assessed using the following frequency bands: very-low frequency (VLF): DC-0.004 Hz, low frequency (LF): 0.004-0.6 Hz and high frequency (HF): 0.6-2.4
Hz [18]. Calculations considered the relative power of the LF and HF powers expressed as normalized units and the LF/HF ratio.

**Carotid body chemosensory recordings**

The CB chemosensory discharge was measured as previously described [11]. Briefly, one carotid sinus nerve was dissected and placed on a pair of Pt electrodes and covered with warm mineral oil. The neural signal was pre-amplified (Grass P511, USA), filtered (30–500Hz) and fed to an electronic spike-amplitude discriminator allowing the selection of action potentials of given amplitude above the noise. Selected action potentials were counted with a frequency meter to assess the CB chemosensory frequency of discharge \( f_x \), expressed in Hz. In the CB recordings, the contralateral carotid sinus nerve was cut to prevent vascular and ventilatory effects caused by the activation of chemosensory reflexes (Supplementary data). The chemosensory discharge was measured at several isocapnic levels of PO\(_2\) (5 to 670 mmHg), maintained until the response was in semi steady state (~10-20 s). Rats breathed spontaneously during the whole experiments. At the end of the experiments, rats were killed by an overdose of pentobarbitone (100 mg kg\(^{-1}\) i.p).

**Lipid peroxidation assay**

A spectrophotometric assay for detection of thiobarbituric acid reactive substances (TBARS) was used as an oxidative stress marker. Blood samples were collected through the carotid artery, and placed in heparinized ice-cold microcentrifuge tubes.
Plasma was separated by centrifugation and stored at –80°C. MDA was used as a standard, and the level of TBARS was reported as nmoles/ml of MDA.

Immunohistochemical detection of 3-NT and iNOS in the carotid body

Anesthetized rats were perfused intracardially with phosphate buffer saline (PBS) at pH 7.4 for 10 min followed by buffered 4% paraformaldehyde (Sigma, USA). Carotid bifurcations containing the CB were dissected and post-fixed in the same fixative solution, dehydrated in ethanol, included in paraffin, cut in 5 µm sections and mounted on silanized slides. Deparaffinized samples were incubated with H$_2$O$_2$ to inhibit endogenous peroxidase and then in blocking serum solution (Vector Lab, USA). The slices were incubated with an anti-3-NT polyclonal antibody (1:20 in PBS/BSA 1%; Molecular Probes A-21285, USA) or an anti-iNOS polyclonal antibody (1:200 in PBS/BSA 1%; Sigma Nº 7782, USA) overnight at 4ºC. Slices were incubated with an universal biotinylated secondary antibody followed by a ready-to-use stabilized ABC reagent (Vectastain Elite ABC Kit, Vector Lab, USA), and revealed with 3,3-diaminobenzidine tetrachloride (Sigma, USA). Samples were counterstained with Harris’ hematoxylin and mounted. Photomicrographs were taken at 100x with a CCD camera coupled to an Olympus CX 31 microscope (Olympus Corp, Japan), digitized and analyzed with the ImageJ software (NIH, USA). The immunoreactive intensity, averaged from 8 fields for each sampled CB, was expressed as optical integrated intensity.

Statistical data analysis
Data was expressed as mean±SEM. Paired comparisons between two groups were performed with the Student test, and differences between more groups were assessed with one or two-way ANOVA tests, followed by Newman-Keuls posthoc comparisons.
RESULTS

The effects of CIH on mean BP, F_H, V_l, V_T, Fr, and basfx measured at the beginning of the recordings while rats breathed spontaneously room air are summarized in Table 1. Mean BP was higher in the CIH-treated rats than in the other groups (p≤0.05), but heart rate did not differ between any groups. Basal V_T, V_l, and Fr in CIH-rats were not significantly different from those recorded from Sham, CIH-AA or Sham AA-rats. However, basfx was significantly higher (p≤0.05) in rats exposed to CIH (80.7 ± 11.4 Hz, n=11) than basfx measured in Sham rats (47.8 ± 5.6 Hz, n=8). Treatment with AA prevented the increased of basfx in rats exposed to CIH.

Rats exposed to CIH showed higher ventilatory responses as compared with Sham, CIH-AA, and Sham-AA rats (fig. 1, p≤0.001, two-way ANOVA). The CB chemosensory response to acute hypoxia was also enhanced by CIH (figs. 1b and 2). The two-way ANOVA analysis indicated that the overall CB chemosensory curve for PO2 was different in CIH rats (p≤0.001) compared with the other groups. The posthoc test indicates that CB chemosensory discharges were higher (p≤0.01) not only in the hypoxic range, but also in normoxia. The AA treatment prevented the enhanced ventilatory and CB chemosensory responses induced by CIH.

As is shown in Table 1, CIH produced a significant increase of MABP, while AA treatment prevented the increase in MABP in CIH rats. In addition, CIH produced a marked modification of the distribution of the relative spectrum power of LF and HF frequency bands, which was prevented by AA (figs. 3 and 4). Figure 4 shows the
summary of CIH effects on LF/HF ratio, LF and HF bands. Rats exposed to CIH had a significant higher LF/HF ratio (0.56 ± 0.08) than the other groups (fig. 4a) due to a reduction of the HF band and a concomitant increase of the relative power of the LF band (fig. 4b-c). We found a statistically significant linear correlation (r≥0.45, p<0.05) between the ventilatory response to 5% inspired O₂ and LF/HF ratio only in the CIH-treated rats. On the contrary, in the other groups, the correlation coefficients were low and lacked statistical significance (Supplementary data).

We found a significant increase of 3-NT-ir (fig. 5) and iNOS-ir (fig.6) in the CB from CIH rats. The 3-NT-ir and iNOS-ir were found in chemoreceptor cell clusters and in the endothelium of interlobular vessels (figs. 5-6). The relative expression level of 3-NT-ir was lower in the CBs from CIH-AA than that of CIH rats. Negative controls, omitting the primary antibody were consistently devoid of staining (not shown). Figure 7 shows the quantification of the effects of CIH on 3-NT-ir (fig. 7a), iNOS-ir (fig. 7b) and plasma MDA (fig. 7c). The CIH–treated rats showed 2.5 fold increase in plasma MDA levels than Sham, CIH-AA and Sham AA–treated rats (p≤0.01, one-way ANOVA). Rats exposed to CIH, but treated with AA showed a 70% of reduction in MDA plasma level as compared with the CIH rats.
Discussion

The main finding of this study shows that AA, which reduced the increased MDA plasma level and the 3-NT-ir in the CB, prevented the potentiation of CB chemosensory and ventilatory responses to acute hypoxia, as well as HRV alterations and the hypertension induced by CIH. Thus, present results support the proposal that oxidative stress plays an essential role in the potentiation of CB chemosensory responses to acute hypoxia, as well as in the cardiorespiratory alterations induced by CIH.

A growing body of evidence supports the idea that an enhanced CB chemosensory responsiveness contributes to the progression of the cardiorespiratory alterations induced by OSA. Fletcher et al. [5] were the first to propose that the CB contributes to the hypertension induced by CIH, because they found that bilateral CB denervation prevented the increased sympathetic discharges and the hypertension in rats exposed to CIH for 35 days. This proposal had received further support since recordings of CB chemosensory discharges showed that CIH augments CB chemosensory discharges in normoxia and hypoxia [10,11,14], and potentiates ventilatory responses to hypoxia [11,12,19].

The alteration of the autonomic balance resulting from the enhanced hypoxic CB chemosensitivity is thought to be involved in the HRV alterations, the reduction of the efficiency of the baroreflex control of heart rate, and the hypertension in OSA patients [20,21] and animals exposed to CIH [11,18,22]. Present results confirmed our previous observation that cyclic hypoxic stimulation of the CB induces selective alterations of HRV [11,22]. LF and HF bands are related to autonomic control on the heart rate. While
the HF band has been associated mainly to cardiac parasympathetic efferent activity, the LF band is believed to be modulated mostly by the sympathetic tone [21]. Recently, Lai et al. [18] found that the LF/HF ratio measured from BP was significantly higher in CIH-exposed conscious rats, related to control rats kept under normoxic condition. They found a significant increase of LF/HF started 5 days after onset of the CIH exposure that lasted until the 30 days of the experiment. The elevation of LF/HF ratio was due to an increase of the LF band, without significant changes of HF. Similarly to their findings, present data show that CIH increased LF/HF mainly due to an increased power of the LF band, but we also found a significant reduction of the HF power, suggesting that CIH modified the autonomic balance of heart rate, with a relative predominance of the sympathetic over parasympathetic activity. Moreover, we found that CIH-induced increase in the LF/HF ratio significantly correlates with the enhanced ventilatory response to acute hypoxia (see supplementary data). Patients with long or recently diagnosed OSA show an increased LF/HF ratio, with a relative predominance of the LF band and a reduced contribution of the HF band [20]. Thus, rats exposed to CIH in this study, similarly to OSA patients, show an increased LF/HF ratio suggesting the existence of changes in the autonomic control of heart rate.

Some methodological issues are needed to discuss. We used a CIH pattern consisting in 12 episodes h⁻¹ (5%O₂ for 20s) instead of the protocol of 30 episodes h⁻¹ (10% O₂ for 90s) utilized in rodents [19], because it is well established that the former pattern enhances the CB chemosensory response to acute hypoxia in rats [11] and cats [20]. Although, it is possible that the 30 episodes h⁻¹ pattern may evoke different results, it is worth to note that also enhances the ventilatory responses to hypoxia in rats [19],
suggesting that the CB chemoreflex responses to hypoxia is potentiated. One limitation of the present study is the use of anesthesia necessary to record CB chemosensory discharges. Since anesthesia may depress cardiorespiratory reflexes and sympathetic outflow [23], we cannot preclude a depressor effect of pentobarbitone on ventilatory and cardiovascular responses. Nonetheless, clinical assessment of the depth of anesthesia did not show apparent differences among the experimental groups.

Peng et al. [13,15] found evidence of the involvement of ROS in the potentiation of CB chemosensory responses to hypoxia induced by CIH. They reported that pretreatment of rats for 10 days before CIH exposure the superoxide dismutase mimetic manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP) prevents the CB chemosensory facilitation induced by repetitive hypoxia in CBs from rats exposed to CIH. However, when MnTMPyP was applied 15 min after the onset of the chemosensory facilitation, failed to block the following increased chemosensory discharges. On the contrary, catalase (an H2O2 scavenger) applied 15 min after the onset of the chemosensory facilitation blocked the increased chemosensory discharges. These observations suggest that O2·− and H2O2 may contribute to the CB chemosensory potentiation induced by CIH, but it is not known whether CIH increases ROS levels in CB chemoreceptor cells or in endothelial cells. Peng et al. [13,15] proposed that ROS may enhance CB chemosensitivity by modifying the O2-sensitive of K+ channels in chemoreceptor cells, producing a large membrane depolarization, which in turn increases the intracellular Ca2+ and the release of excitatory transmitters [24]. However, a direct excitatory effect of ROS on CB chemoreception is matter of debate [25]. An alternative explanation is that an excessive ROS level may impair the mitochondrial
metabolism, similarly to what is observed when the CB is stimulated with large amounts of NO [26]. Present results showing a marked increase of iNOS-ir and 3-NT-ir in the CB tissue suggest that CIH may increase the NO production. It is known that iNOS is responsible for the overproduction of NO in several tissues including the CB of rats exposed to chronic sustained hypoxia [27]. NO reacts with superoxide radical to form peroxynitrite, which undergoes heterolytic cleavage to form hydroxyl anion and nitronium ion, the latter of which nitrates protein tyrosine residues. The treatment with AA prevented the increase of 3-NT-ir in the CB of CIH-rats, but did not reduce the increased iNOS-ir, suggesting that changes in iNOS expression are not directly dependent on the CIH-induced oxidative stress. Since the hypoxic inducible factor 1α (HIF-1α) plays a key role in the CB potentiation induced by CIH [28], it is likely that the high iNOS-ir found in CBs from CIH and CIH-AA rats, could be the result of a direct activation of the HIF-1α transcription pathway by the cyclic hypoxic pattern rather than by the oxidative stress. Our results suggest that iNOS plays a role in the mechanisms mediating the nitrotyrosine residue formation in the CB, which in turn may lead to an excessive NO production and functional losses associated with the chemosensory potentiation to hypoxia [26]. Li et al [29] found that both iNOS expression and activity were upregulated by CIH hypoxia in mice, leading to significant peroxynitrite formation, neuronal injury and spatial memory deficit. In addition, Zhan et al. [30] reported that transgenic absence of iNOS or pharmacological inhibition in mice confers resistance to CIH-induced hypersomnolence and sleepiness, and protection against brain lipid peroxidation and proinflammatory gene overexpression. Thus, the available data support a critical role for iNOS in the development of the CIH-induced oxidative stress and inflammatory responses, suggesting a potential role for inducible NO in OSA.
Few experimental studies have addressed the contribution of oxidative stress on the CIH-induced hypertension. The finding that AA prevents the hypertension and HRV alterations in CIH-rats confirm and extend previous observations showing that daily intraperitoneal administration of MnTMPyP abolished the hypertension and the increased plasma catecholamines in rats exposed to CIH [17]. In addition, our results showing that AA prevents the potentiation of the ventilatory responses agree with the observation of MacFarlane and Mitchell [13], who found that MnTMPyP application into the intrathecal space of the cervical spinal cord, abolished the phrenic long-term potentiation induced by acute intermittent hypoxia in rats. Thus, ROS formation seems to be needed for CB and motor respiratory plasticity induced by CIH.

Our results support a preventing role for AA supplementation on the oxidative stress, the potentiation of CB chemoreflex as well as on the development of HRV alterations and hypertension induced by CIH. Thus, a potential therapeutic effect of antioxidants in OSA deserves further attention. However, a relevant concern is the human equivalence of the AA dose used in this study. This is a crucial question that needs to be addressed before any clinically study can be achieved in humans. Scaling doses from animal studies to humans is a complex topic. The animal effective dose should not be extrapolated to the human equivalent dose (HED) by a simple conversion based on body weight. Contrarily, a normalization based on body surface area (BSA) seems to be more appropriate [31]. If we assume that a 200 g rat drinks about 10 ml/day of water containing 1.25 g/l AA, the daily-consumed amount is about 62 mg/kg. The HED formula for the dose translation based on BSA is calculated by multiplying the animal dose for the ratio of animal to human weight/BSA scaling factors. [31]. Accordingly, if we translate the animal dose
used here to the HED using the published scaling factors of 6 and 37, respectively for a 150 g rat and a 60 kg human, the HED dose is 10 mg/kg, corresponding to a daily total of 600 mg AA. The only data published in humans are those by Schulz et al. [32], who demonstrated evidence of oxidative stress in OSA patients and improved flow-mediated vasodilatation in the brachial artery after acute infusion of 500 mg AA. Thus, the positive effects of antioxidants in OSA may extend beyond the effects on the CB.

In summary, present results show a preventive role for AA in the generation of oxidative stress, potentiation of CB chemoreflexes as well as on the development of HRV and BP alterations induced by CIH, supporting an essential role for systemic and local CB oxidative stress on the progression of the cardiorespiratory alterations induced by CIH.
Support statement

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References


Fig. 1. Effects of ascorbic acid (AA) on ventilatory (a) and carotid body chemosensory (b) responses to several levels of inspired PO$_2$ in Sham rats (------ and ○), CIH exposed rats (—— and ●), Sham rats treated with ascorbic acid (------ and △) and rats exposed to CIH treated with ascorbic acid (—— and ▲). $V_i$: ventilatory minute expressed as % of normoxia in 22 CIH rats, 20 Sham, 11 CIH AA and 8 Sham AA rats. $f_x$: carotid chemosensory frequency of discharge in Hz measured in 11 CIH rats, 8 Sham, 10 CIH AA and 4 Sham AA rats. ***: p≤0.001; **: p≤0.01; and *: p≤0.05 compared to sham control.
Fig. 2. Representatives recordings of carotid body chemosensory responses to brief hypoxic stimulus (fill bar: inspired PO$_2$ ~ 5 mmHg) in one CIH-treated rat (a), one Sham (b) and one CIH AA-treated rats (c). $f_x$, carotid chemosensory frequency of discharge in Hz.

Fig. 3. Representative traces of the power spectral density of heart rate variability in one Sham rat (Sham), one rat exposed to CIH (CIH), one Sham rat treated with ascorbic acid (Sham AA) and in one CIH rat treated with ascorbic acid (CIH AA). PDS: Power spectral density expressed in normalized units (n.u.).
Fig. 4. Spectral indexes of R-R variability in Sham rats (Sham, n=10), CIH exposed rats (CIH, n=24), Sham rats treated with ascorbic acid (Sham AA, n=11) and CIH rats treated with ascorbic acid (CIH AA, n=10). a) LF/HF, low/high ratio of power spectral density, b) LF, low frequency band expressed in normalized units (n.u.), c) HF, high frequency band, expressed in normalized units (n.u.). ***: p ≤ 0.001; and **: p ≤ 0.01 compared to sham; #: p ≤ 0.01; and +: p ≤ 0.05 compared to CIH.
Fig. 5. Micrographs showing positive immunoreactive for 3-Nitrotyrosines (3-NT-ir) in CBs from Sham rat (a), CIH exposed rat (b), and CIH rat treated with ascorbic acid (c). Left panel, immunohistochemistry. Right panel: Images after colour deconvolution for quantification of 3-NT-ir. Scale bars 20 µm.

Fig. 6. Micrographs showing positive immunoreactive for inducible nitric oxide synthase (iNOS-ir) in CBs from Sham rat (a), CIH exposed rat (b) and a CIH rat treated with
ascorbic acid (c). Left panel, immunohistochemistry. Right panel, Images after colour deconvolution for quantification of iNOS-ir. Scale bars 20 µm.

Fig. 7. Summary of the effects of ascorbic acid (AA) on CB and systemic oxidative stress induced by CIH. (a) Nitrotyrosines immunoreactivity (3-NT-ir) and (b) inducible nitric oxide synthase (iNOS-ir) immunoreactivity measured in CBs from 6 Sham, 8 CIH and 5 CIH-AA rats. Data presented as percentage of Sham immunoreactivity. (c), Malondialdehyde (MDA) plasma levels in 14 Sham, 10 CIH and 6 CIH AA rats. ***: p≤0.001 versus sham; ‡: p≤0.001 versus CIH.
Table 1. Effect of CIH on physiological variables at normoxia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>CIH</th>
<th>Sham AA</th>
<th>CIH AA</th>
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<tr>
<td>MABP (mmHg)</td>
<td>111 ± 4 (n= 15)</td>
<td>138 ± 3*** (n= 16)</td>
<td>117 ± 6# (n= 9)</td>
<td>109 ± 6‡ (n= 10)</td>
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<tr>
<td>FH (bpm)</td>
<td>427 ± 17 (n= 15)</td>
<td>403 ± 14 (n= 16)</td>
<td>394 ± 15 (n= 9)</td>
<td>386 ± 17 (n= 10)</td>
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<td>VT, (ml/kg)</td>
<td>4.4 ± 0.5 (n= 14)</td>
<td>3.6 ± 0.4 (n= 22)</td>
<td>3.4 ± 0.2 (n= 11)</td>
<td>3.6 ± 0.2 (n= 11)</td>
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<tr>
<td>Fr, (bpm)</td>
<td>78± 6 (n= 14)</td>
<td>74 ± 3 (n= 22)</td>
<td>80 ± 4 (n= 11)</td>
<td>78 ± 3 (n= 11)</td>
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<tr>
<td>VI, (ml/min kg)</td>
<td>369 ± 58 (n= 14)</td>
<td>273 ± 37 (n= 22)</td>
<td>286 ± 26 (n= 11)</td>
<td>291 ± 39 (n= 11)</td>
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<tr>
<td>bas fx, (Hz)</td>
<td>48 ± 6 (n= 8)</td>
<td>81 ± 11** (n= 11)</td>
<td>42 ± 4# (n= 5)</td>
<td>47 ± 4# (n= 10)</td>
</tr>
</tbody>
</table>

Data are presented as mean± SEM. Sham: control animals; CIH: rats exposed to chronic intermittent hypoxia; Sham AA: control rats that received oral ascorbic acid; CIH AA: rats supplemented with oral ascorbic acid during the exposure to chronic intermittent hypoxia; MABP, mean arterial blood pressure; FH: heart frequency; VT: tidal respiratory volume; Fr: respiratory frequency; VI: minute ventilatory volume; bas fx: basal carotid body chemosensory activity: ***: p≤0.001 versus sham; **: p≤0.01 versus sham; ‡: p≤0.001 versus CIH; #: p≤0.01 versus CIH.