Chronic intermittent hypoxia enhances cat chemosensory and ventilatory responses to hypoxia

Sergio Rey[1], Rodrigo Del Rio[1], Julio Alcayaga[2] and Rodrigo Iturriaga[1]

[1]*Laboratorio de Neurobiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile*
[2]*Laboratorio de Fisiología Celular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile*

The carotid body (CB) chemoreceptors may play an important role in the enhanced hypoxic ventilatory response induced by chronic intermittent hypoxia (CIH). We studied the effects of cyclic hypoxic episodes of short duration on cat cardiorespiratory reflexes, heart rate variability, and CB chemosensory activity. Cats were exposed to cyclic hypoxic episodes ($P_{O_2} \sim 75$ Torr) repeated during 8 h for 2–4 days. Cats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1} I.P., followed by 8–12 mg I.V.), and ventilatory and cardiovascular responses to NaCN (0.1–100 μg kg^{-1} I.V.) and several isocapnic levels of oxygen ($P_{O_2} \sim 20$–740 Torr) were studied. After studying the reflex responses, we recorded the CB chemosensory responses induced by the same stimuli. Results showed that CIH for 4 days selectively enhanced cat CB ventilatory (V_T and V_I) responses to hypoxia, while responses to NaCN remained largely unchanged. Similarly, basal CB discharges and responses to acute hypoxia ($P_{O_2} < 100$ Torr) were larger in CIH than in control cats, without modification of the responses to NaCN. Exposure to CIH did not increase basal arterial pressure, heart rate, or their changes induced by acute hypoxia or hyperoxia. However, the spectral analysis of heart rate variability of CIH cats showed a marked increase of the low-/high-frequency ratio and an increase of the power spectral distribution of low frequencies of heart rate variability. Thus, the enhanced CB reactivity to hypoxia may contribute to the augmented ventilatory response to hypoxia, as well as to modified heart rate variability due to early changes in autonomic activity.

Exposure of humans and animals to sustained hypoxia for hours or days is usually known as chronic hypoxia. However, cyclic hypoxic episodes of short duration followed by normoxia, termed chronic intermittent hypoxia (CIH), have received much attention in recent years (Fletcher, 2000; Sica *et al.* 2000; Prabhakar *et al.* 2001; Prabhakar & Peng, 2004). This pattern of CIH is a characteristic of pathological conditions such as obstructive sleep apnoea (OSA). CIH appears to enhance the hypoxic ventilatory response (HVR), leading to hypertension and upregulation of catecholaminergic and renin–angiotensin systems (Fletcher, 2000; Sica *et al.* 2000; Prabhakar *et al.* 2001). Most of the information on the effects of CIH on peripheral chemoreflex control of cardiovascular and respiratory systems has been obtained from studies performed on patients with OSA. However, conclusions from these studies are conflicting because of concomitant effects of morbidities associated with OSA (Narkiewicz *et al.* 1999). More recently, experiments

performed in animal models showed that CIH enhances HVR (Ling *et al.* 2001) and produces long-term facilitation of respiratory motor activity (McGuire *et al.* 2003; Peng & Prabhakar, 2003). The facilitatory effect of CIH on HVR has been attributed to a potentiation of carotid body (CB) chemosensory responses to hypoxia. However, it is a matter of debate whether the effects induced by CIH on HVR are due to a CB-enhanced activity or secondary to central facilitation of chemosensory input (Mitchell *et al.* 2001). Recently, Peng *et al.* (2001, 2003) found that basal CB discharges and chemosensory responses to acute hypoxia were larger in rats exposed to a pattern of CIH of short cyclic hypoxic episodes followed by normoxia, applied for 8 h each day for 10 days. Nevertheless, this observation has not been confirmed in other animal models of CIH. The ventilatory response to hypoxia does not only depend on the pattern, duration and intensity of hypoxic stimuli, but species differences may also account for the reported disparities

in time course and excitatory or inhibitory effects of hypoxia on ventilation (Powell *et al.* 1998, 2000). Using a protocol of short hypoxic episodes, we examined the early effects of CIH (2–4 days) on cat cardiorespiratory reflexes and CB chemosensory responses induced by hypoxia, and compared them with the responses elicited by NaCN.

Methods

Animals

Experiments were performed on 19 male adult cats (2.0–4.7 kg). The experimental protocol was approved by the Ethical Committee of the Facultad de Ciencias Biológicas of the Pontificia Universidad Católica de Chile, and was performed according to the Guiding Principles for the Care and Use of Animals of the American Physiological Society.

Exposure to multiple short episodes of chronic intermittent hypoxia (CIH)

Nine awake male cats (3.35 ± 0.26 kg; mean ± s.e.m.) were housed in a Plexiglas cylindrical chamber, with internal dimensions of 30 cm in diameter and 60 cm long, yielding a volume of 35.2 l. The chamber was alternately flushed with 100% N_2 (13 l min^{-1}) for 90 s or compressed air (20 l min^{-1}) for 270 s, using timed solenoid valves. This gas alternation in the chamber produced a see-saw pattern of P_{O_2}, which dropped to a minimum of ∼75 Torr in about 100 s and then slowly returned to near 150 Torr, reducing the P_{O_2} below 125 and 100 Torr for 140 and 60 s, respectively (Fig. 1). The intermittent hypoxic pattern was repeated 10 times an hour for 8 h day^{-1} for 2 days (2 cats) or 4 days (7 cats). The P_{O_2} in the chamber was monitored
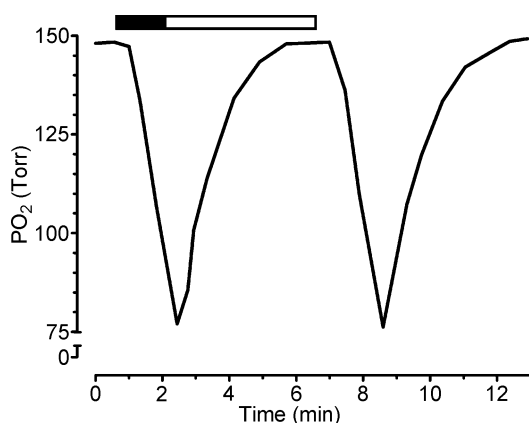

Figure 1. Changes of P_{O_2} levels in the chamber induced by the hypoxic challenge
The hypoxic pattern consisted of 90 s of 100% N_2 (■) followed by 270 s of room air (□), repeated 10 times h^{-1} 8 h day^{-1} for 4 days. P_{O_2} reached a minimum of about 75 Torr during hypoxia. The level of P_{O_2} in the chamber was monitored with an oxygen analyser.

using an oxygen analyser (Ohmeda 5120, Madison, WI, USA). Acute experiments were performed the morning of the day after the end of hypoxic exposures. As a control group, ten male cats (3.62 ± 0.22 kg) were kept in normoxic conditions; two of them were subjected to a similar pattern of gas dynamics in the chamber, replacing N_2 by compressed air. We did not find any difference in the basal ventilatory and cardiovascular variables and in the responses to hypoxia (one-way ANOVA, $P > 0.05$) between these latter two groups, so their data were pooled together as the control group.

Recording of physiological responses

Cats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1} I.P.), additional doses (8–12 mg I.V.) were given when necessary to maintain a level of surgical anaesthesia (stage III, plane 2) assessed by the absence of withdrawal reflexes to strong pressure on the paws, with persistence of patellar reflexes. Cats were placed in supine position and the body temperature was continuously monitored through a thermistor probe introduced into the rectum, and maintained at 38.0 ± 0.5°C with a heated pad. The trachea was cannulated with a flexible tube for recording the airflow signal with a pneumotachograph (Fleish number 00, Lausanne, Switzerland). A fine tube was introduced into the tracheal cannula for continuous sampling of the tracheal CO_2 pressure (P_{ET,CO_2}) through a capnograph (Poet II Criticare CSI, WI, USA). One saphenous vein was cannulated for administration of drugs. Arterial blood pressure was recorded from one femoral artery through a cannula filled with 50 IU ml^{-1} of heparin in saline solution, and connected to a pressure transducer (Statham P23, Oxnard, CA, USA). The ECG was continuously recorded using the II lead. All the physiological variables were fed to an analog-digital system (PowerLAB/8SP, AD Instruments, Castle Hill, Australia), connected to a computer. Minute inspiratory volume (V_I), tidal volume (V_T) and respiratory frequency (f_R) were digitally obtained from the airflow signal. Heart rate (f_H) was obtained from the ECG, and mean arterial pressure (BP) from the arterial pressure signal. The physiological data were analysed using Chart 5.0 software (AD Instruments, Castle Hill, NSW, Australia). To assess the effects of CIH on sensitivity and reactivity of peripheral chemoreceptor reflexes, we studied the cardiovascular (BP, f_H) and ventilatory (V_I, V_T, f_R) responses elicited by several isocapnic levels of P_{O_2} (∼20–740 Torr) maintained during 1–2 min and by I.V. injections of NaCN (0.1–100 µg kg^{-1}).

Carotid body chemosensory recordings

After finishing the study of cardiorespiratory reflexes, the carotid chemosensory discharge was recorded in some

cats as previously described (Iturriaga *et al.* 1998). One carotid sinus nerve and the ipsilateral ganglio-glomerular nerves were dissected and cut. After the excision of the epineurium, the carotid sinus nerve was placed on a pair of platinum electrodes and covered with warm mineral oil. The neural signal was pre-amplified (Grass P511, West Warwick, RI, USA), with a bandwidth of 30 Hz–1 kHz, amplified, filtered (notch filter of 50 Hz), and fed to an electronic spike-amplitude discriminator. This procedure allows the selection of action potentials of given amplitude above the noise to be counted with a frequency meter for assessing the frequency of CB chemosensory discharge (f_x), expressed in Hz. Carotid sinus barosensory fibres were eliminated by crushing the common carotid artery wall between the carotid sinus and the CB. The chemosensory discharge was measured at several isocapnic levels of P_{O_2} ($\sim$20–740 Torr), maintained for 1–2 min, and during I.V. injections of NaCN (0.1–100 μg kg^{-1}). Cats breathed spontaneously during the experiments. We did not neuromuscularly block cats because cholinergic blockers impair CB chemosensory chemoreception to hypoxia (Jonsson *et al.* 2000). In some experiments, the contralateral carotid sinus nerve was also cut to prevent vascular and ventilatory effects caused by the activation of chemosensory reflexes. At the end of the experiments, cats were killed by an overdose of sodium pentobarbitone (120 mg I.V.) administered until the ventilation and ECG were completely abolished and the arterial pressure decreased to zero.

Spectral analysis of heart rate variability

Under anaesthesia and prior to any manoeuvre or drug injection, the ECG was recorded for 5 min at 2 kHz. After acquisition of the ECG, the signal was visually explored for detection of ectopic QRS complexes and the RR intervals were measured and converted to ASCII format. The heart rate variability was analysed with software created by The Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland (Tarvainen *et al.* 2002; Niskanen *et al.* 2004). This program was originally designed to analyse human RR interval data; however, cats have higher heart and respiratory rate, which account for different peak frequencies observed in the spectral analysis derived from the RR intervals (Table 2). Thus, we used wider frequency intervals, according to a previously published study in the cat (Di Rienzo *et al.* 1991). To assess the heart rate variability, RR interval data were analysed with frequency domain methods. The most common frequency domain methods for heart rate variability analysis are the Fast Fourier Transform (FFT) algorithm and autoregressive methods. In this study, the statistical comparisons and calculations were derived from FFT data, although we also used autoregressive method for illustration of the

main oscillatory components of heart rate variability in graphics. The order of the autoregressive fit was adjusted according to FFT data obtained from the ECG. Despite the fact that FFT calculations are more accurate than autoregressive models, the latter can be used for parametric estimation of the main characteristics of the power spectral density of heart rate variability, specially to resolve peak frequencies and to display a more normalized shape in the power graphics. The model order for autoregressive data analysis varied from 13 to 20. To reduce complex linear or non-linear trends in the RR interval signal, we used a detrending algorithm based on the smoothness priors method (Tarvainen *et al.* 2002). Processing the signal with this method reduces the power spectral density in the VLF range ($<$0.025 Hz), specially below the cut-off frequency (0.016 Hz). The very-low-frequency modulation of RR interval data has no biological interpretation in short ECG recordings, and could alter the estimation of the low-frequency component (Task Force, 1996). Spectral data obtained from RR intervals were analysed according to the following frequency bands: very-low frequency (VLF, 0.000–0.025 Hz), low frequency (LF, 0.025–0.140 Hz), and high frequency (HF, 0.140–0.600 Hz) bands (Di Rienzo *et al.* 1991). We used the LF/HF ratio to measure the autonomic influences on heart rate variability (Task Force, 1996). Calculations considered the total power of heart rate variability, the relative power of the three frequency bands expressed as percentages of total power, and LF and HF powers expressed as normalized units (which consider the percentage of total power minus the VLF component), and the LF/HF ratio.

Statistical analysis

Results were expressed as mean $\pm$ S.E.M. Ventilatory and CB chemosensory discharges measured at different levels of P_{O_2} were fitted to the following exponential curve:

$$R = R_{bas} + \left[R_{max} - R_{bas} \right] e^{(-K P_{O_2})}$$

where R = response, R_{bas} = basal activity, R_{max} = maximal response, and K = decay constant. To compare the effects of CIH on the maximal reactivity and on the ED$_{50}$ of dose–response curves induced by NaCN, data were fitted to the logistic expression:

$$R = R_{bas} + \{ [R_{max} - R_{bas}] / [1 + (ED_{50}/D)^S] \}$$

where D = dose, and S = Hill slope factor determining the steepness of each curve. The correlation coefficients for all adjusted curves were $>$0.95 ($P < 0.01$) for all conditions studied. Statistical differences between responses measured at different P_{O_2} levels and dose–response curves of control *versus* CIH cats were analysed by a two-way ANOVA, and *post hoc* analyses were performed by the Bonferroni test. Differences for three or two samples

Table 1. Effects of four days of chronic intermittent hypoxia (CIH) on the baseline values of physiological variables measured at the beginning of the experiments

	$f_{x,bas}$ (Hz)	f_R (min^{-1})	V_T (ml kg^{-1})	V_I (ml min^{-1} kg^{-1})	P_{ET,CO_2} (Torr)	BP (Torr)	f_H min^{-1}
Control	36.0 ± 5.1	30.9 ± 1.4	7.9 ± 0.5	253.4 ± 15.4	28.5 ± 1.0	129 ± 4.3	239.2 ± 11.7
CIH	63.2 ± 7.3*	23.9 ± 3.4*	9.9 ± 0.8	249.3 ± 23.5	27.6 ± 0.8	122.9 ± 6.9	212.6 ± 12.0

All physiological variables were measured in normoxic conditions. $f_{x,bas}$, basal frequency of CB chemosensory discharge, measured for 60 s *$P < 0.01$, statistically different from control. Student's t test. $n = 10$ cats in control and 7 CIH cats, except $n = 6$ in both groups for f_x recordings.

were assessed by means of one-way ANOVA or Student's unpaired t test, respectively. The level of significance for the statistical analyses was $P < 0.05$.

Results

Effects of chronic intermittent hypoxia on basal variables

Table 1 shows the effects of a 4-day CIH exposure on basal levels of ventilatory and cardiovascular variables measured at the beginning of the experiments in seven cats. Basal V_T, V_I, P_{ET,CO_2}, BP, and f_H in CIH cats were not significantly different from those observed in ten control cats ($P > 0.05$) and were similar to those previously reported in cats anaesthetized with sodium pentobarbitone (Oyarzun *et al.* 1991; Iturriaga *et al.* 1998). Despite the fact that CIH-treated cats had lower basal f_R ($P < 0.01$), basal V_I was unchanged ($P > 0.05$) due to a larger, although not statistically significant V_T in the CIH group. The $f_{x,bas}$ measured in normoxic conditions at the beginning of the carotid sinus nerve recordings was significantly higher ($P < 0.05$; Student's unpaired t test) in cats exposed to CIH for 4 days (63.2 ± 7.3 Hz, $n = 6$) than $f_{x,bas}$ measured in control cats (36.3 ± 5.1 Hz, $n = 6$).

Effects of chronic intermittent hypoxia on ventilatory responses to acute hypoxia and NaCN

Both control and CIH cats demonstrated a preferential increase in V_T over f_R in response to acute hypoxia and NaCN. The exposure to CIH for 2 days did not modify the cat ventilatory (V_T and V_I) responses elicited by hypoxia or NaCN (data not shown, $n = 2$). However, exposure of cats to CIH for 4 days enhanced the ventilatory responses (V_T and V_I) to acute hypoxia. The effects of CIH on V_I and V_T measured at several P_{O_2} levels (20–740 Torr) are shown in Fig. 2. The curves for the relationships between P_{O_2} and V_I and V_T were significantly different in four CIH cats as compared with six control cats ($P < 0.001$, two-way ANOVA). The Bonferroni test showed that V_I and V_T were statistically higher at a P_{O_2} of about 20 Torr (Fig. 2). On the contrary, CIH did not enhance the

V_T dose–response curve induced by NaCN ($P > 0.05$, two-way ANOVA), and the Bonferroni test did not show significant differences for any specific dose of NaCN between CIH and control animals (Fig. 3). The Hill slope coefficient and the ED$_{50}$ for the adjusted curves were not different between both groups (control *versus* CIH group, Hill slope factor of 1.37 ± 0.35 *versus* 1.47 ± 1.80, and ED$_{50}$ of 15.0 ± 1.3 *versus* 20.9 ± 2.9 μg kg^{-1}, respectively, $P > 0.05$). Moreover, CIH did not change the maximal reactivity of V_T induced by NaCN. Indeed, the $V_{T,max}$ predicted by the logistic fit model for the control group was 22.0 ± 1.5 ml kg^{-1}, which was not statistically different from the $V_{T,max}$ value for the CIH cats of 20.4 ± 4.6 ml kg^{-1} ($P > 0.05$).

Effects of chronic intermittent hypoxia on cardiovascular responses to hypoxia and NaCN, and heart rate variability

Figure 4 summarizes the effects of CIH on the cardio-vascular variables (BP and f_H) measured when cats breathed isocapnic hypoxic, normoxic and hyperoxic gas mixtures (P_{O_2} of 22, 150 and 740 Torr, respectively). Clearly, successive lowering of P_{O_2} from the hyperoxic level increased BP (Fig. 4*A*) and heart rate (Fig. 4*B*, $P < 0.05$, two-way ANOVA) in both experimental groups, but CIH did not enhance the amplitude of the responses with respect to control group ($P > 0.05$, two-way ANOVA). In addition, BP$_{max}$ and $f_{H,max}$ elicited by doses of 100 μg kg^{-1} of NaCN were not different in both groups (data not shown). Thus, CIH did not potentiate the cardiovascular responses elicited by peripheral chemo-receptor stimulation. However, the spectral analysis of heart rate variability showed that CIH produced a marked modification of the distribution of the absolute and relative power of the oscillatory components of heart rate variability, without changing the peak frequencies (Table 2). Figure 5 illustrates the distribution of the spectral components of heart rate variability normalized to the total power in three CIH and three control cats. In the CIH cats, the power spectral density analysis showed a predominance of the low-frequency component over high-frequency, while the opposite situation is observed in
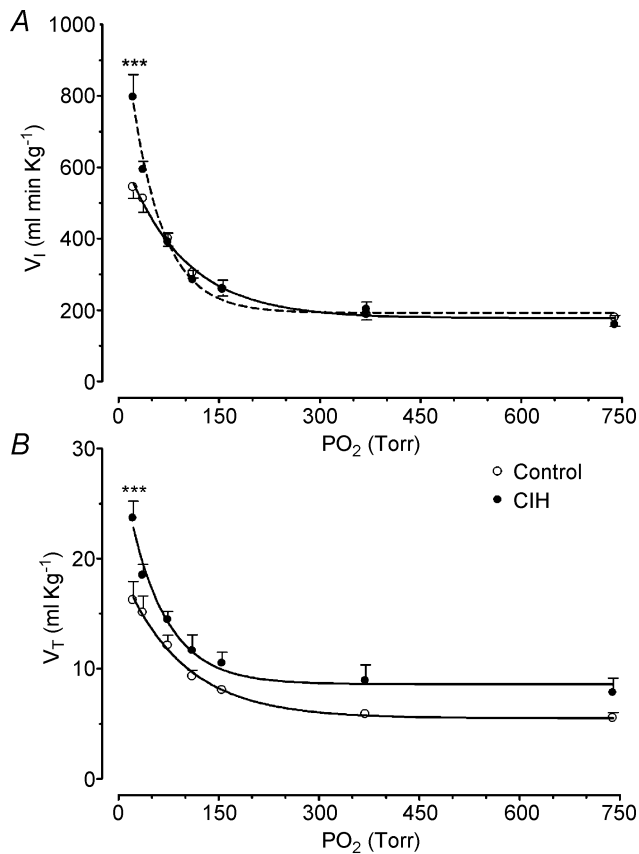
Figure 2. Effects of CIH on ventilatory response to several levels of P_{O_2} in four cats

A, V_I, inspiratory minute volume. B, V_T, tidal volume. ***$P < 0.001$, as compared with six control cats (Bonferroni test after two-way ANOVA). Error bars show S.E.

control animals. As a result of the change in the distribution of the relative spectral components of heart rate variability, cats exposed to CIH showed a significantly higher LF/HF ratio (0.60 ± 0.10 and 2.40 ± 0.10 in control and CIH cats, respectively, $P < 0.001$, Student's *t* test). The effects of CIH
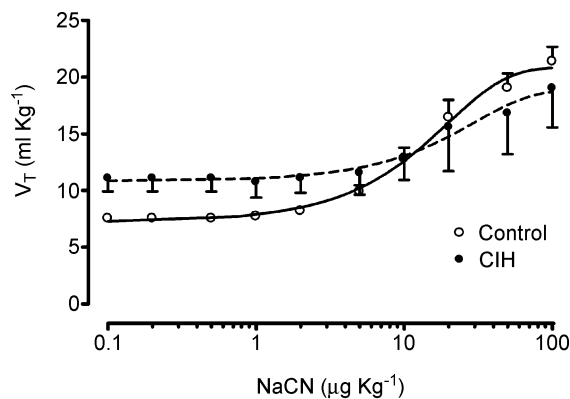

Figure 3. Effects of CIH on V_T dose–response curves to NaCN in four cats

No significant statistical difference between both curves as compared with six control cats (two-way ANOVA and Bonferroni tests). Error bars show S.E.

on the LF/HF ratio and the relative power spectral density distribution of the very-low-, low- and high-frequency bands of the RR interval data are summarized in Fig. 6.

Effects of chronic intermittent hypoxia on CB chemosensory responses to NaCN and hypoxia

Chronic intermittent hypoxic exposure did not modify the CB chemosensory dose–response curve for NaCN ($P > 0.05$, two-way ANOVA, Fig. 7). The Hill slope coefficient and the ED_{50} for the adjusted curves were not different in both groups (control *versus* CIH group, Hill slope factor of 0.08 ± 0.01 *versus* 0.07 ± 0.02, and ED_{50} of 10.0 ± 1.2 *versus* 10.1 ± 2.4 $\mu g\ kg^{-1}$, respectively, $P > 0.05$). CIH did not change the maximal reactivity of f_x induced by NaCN (Fig. 3). The $f_{x,max}$ predicted by the logistic fit model for the control group was 360.0 ± 15.9 Hz and the $f_{x,max}$ value for the CIH cats was 387.7 ± 31.5 Hz ($P > 0.05$). In contrast to what happened with the responses elicited by NaCN, chemosensory
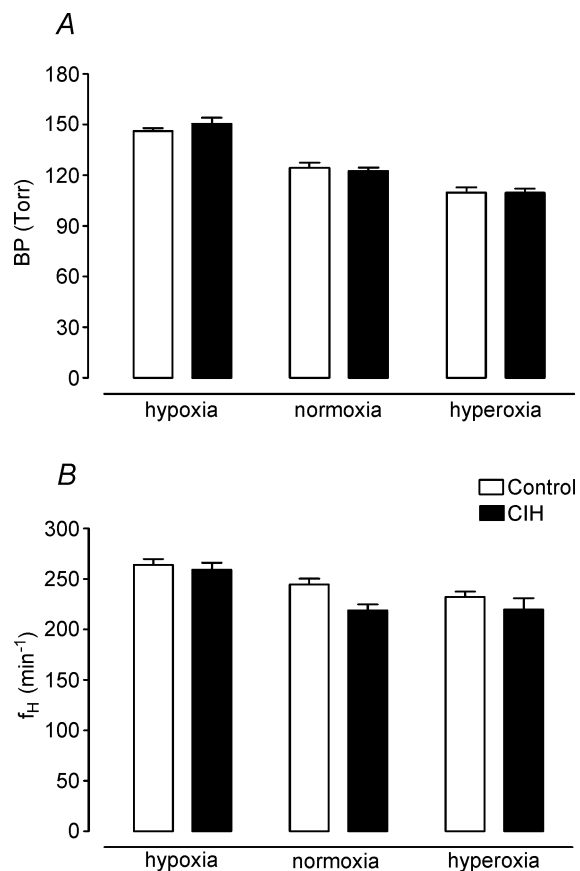

Figure 4. Effects of CIH on mean arterial blood pressure (BP) and heart rate (f_H) at three levels of P_{O_2} in six cats

Lowering P_{O_2} from the hyperoxic level increased BP and heart rate ($P < 0.05$), but CIH did not modify the amplitude of the responses related to six control cats (two-way ANOVA and Bonferroni test). Error bars show S.E.

Table 2. Effects of chronic intermittent hypoxia (CIH) exposure for four days on heart rate variability

	Control	CIH
RR interval (ms)	259.0 ± 28.0	302.0 ± 17.0
Heart rate (min^{-1})	245.0 ± 25.0	202.0 ± 12.0
Peak Frequencies		
VLF (Hz)	0.020 ± 0.001	0.020 ± 0.001
LF (Hz)	0.040 ± 0.010	0.030 ± 0.003
HF (Hz)	0.380 ± 0.06	0.300 ± 0.03
Absolute powers		
VLF (ms^2)	0.02 ± 0.01	0.05 ± 0.01
LF (ms^2)	0.05 ± 0.03	0.15 ± 0.04*
HF (ms^2)	0.11 ± 0.05	0.06 ± 0.01**
Normalized powers		
LF (n.u)	36.0 ± 4.0	70.0 ± 1.0**
HF (n.u)	64.0 ± 4.0	30.0 ± 1.0**

*$P < 0.01$, **$P < 0.001$. Significantly different from control (Student's t test, $n = 6$ cats in control and CIH groups). n.u. normalized units. VLF, very-low-; LF, low- and HF, high-frequency bands.

responses to acute hypoxia were enhanced in the CIH cats as is shown in Fig. 8. The two-way ANOVA analysis showed that the overall chemosensory curve for P_{O_2} was different in CIH cats ($P < 0.001$). The Bonferroni test showed that the chemosensory discharge was higher ($P < 0.001$) in the lowest hypoxic range in CIH cats.

Discussion

The main findings of this study are: (i) cats exposed to a pattern of short hypoxic episodes ($P_{O_2} \sim 75$ Torr) followed by normoxia during 8 h for 4 days showed an enhanced ventilatory response to acute hypoxia, but the ventilatory response induced by NaCN remained the same; (ii) basal CB chemosensory discharges were higher in CIH cats, and CB responses to hypoxia and NaCN showed the same pattern as ventilatory responses; and (iii) CIH did not augment either basal BP and f_H or cardiovascular responses to hypoxia, but the spectral analysis of basal heart rate variability showed a marked increase of the LF/HF ratio. Thus, our results confirm and extend the observations of Peng et al. (2001, 2003), who found that CIH enhances CB chemosensory response to acute hypoxia in rats. Moreover, the present results support the idea that a few days of CIH are enough to produce a plastic change of CB reactivity to hypoxia, which may contribute to the augmented ventilatory response to acute hypoxia, and to early alterations in the autonomic balance, that regulates heart rate variability.

We found that ventilatory and carotid chemosensory responses induced by NaCN were not enhanced by 4 days of CIH. The fact that NaCN, a metabolic poison, increases

chemosensory discharge has supported the idea that the oxidative metabolism of chemoreceptor cells is involved in the O_2 sensing (metabolic hypothesis). However, NaCN stimulates the petrosal neurones that innervate the CB chemoreceptor cells. Indeed, we (Alcayaga et al. 1999) found that NaCN, but not hypoxia, stimulates cat petrosal ganglion neurones, suggesting different mechanisms of action. Thus, CIH may have a different effect on the CB responses elicited by NaCN and hypoxia.

Our results suggest that short-term CIH for 4 days is enough to enhance hypoxic responses in the cat CB, without changing the responses elicited by NaCN. However, we cannot rule out that a prolonged exposure to CIH or a severe hypoxic level may increase the response to other stimuli. Indeed, Prabhakar & Peng (2004) found that 10 days of more severe hypoxic episodes ($\sim$5% O_2 for 15 s followed by 5 min of normoxia, 9 times h^{-1} for 10 days) increases not only rat CB chemosensory responses to acute hypoxia, but also the response induced by NaCN. Similarly, Di Giulio et al. (2003) found that exposure to sustained severe hypoxia ($P_{O_2} \sim 43$ Torr) for 7 h augmented the cat CB chemosensory responses to hypoxia and NaCN.

Ventilatory and chemosensory effects of chronic intermittent hypoxia

Sustained hypoxia increases basal ventilation and HVR in humans and animals, a phenomenon termed ventilatory acclimatization (Bisgard, 2000). Ventilatory acclimatization induced by sustained hypoxia requires the presence of functional CBs, because it does not occur when the CBs are removed (Smith et al. 1986). Sustained hypoxia progressively increases both basal chemosensory discharges and chemosensory responses to acute hypoxia in 4 h in goats (Nielsen et al. 1988) and in 8 h in cats (Di Giulio et al. 2003). In humans, a mild hypoxia maintained for 8 h produces hyperventilation and hypocapnia and increases the reactivity of peripheral ventilatory chemoreflexes to hypoxia (Fatemian et al. 2001). Moreover, Mahamed et al. (2003) found that 3 h of isocapnic-sustained hypoxia produces in humans an enhancement of HVR, due to a decreased threshold of the ventilatory response to CO_2. On the contrary, the effects of intermittent hypoxia on respiratory control and CB chemoreceptor function are less known. According to McGuire et al. (2003), intermittent hypoxia could be classified at least in two types: acute intermittent hypoxia (AIH), characterized by several hypoxic episodes over a short period of time, and CIH, characterized by several hypoxic episodes over a period of days or weeks. The AIH produces long-term facilitation of motor respiratory activity that lasts for several minutes after the end of the periodic of hypoxic challenge (Baker & Mitchell, 2000; Mitchell et al. 2001). In addition to the potentiation of the CB chemosensory responses,
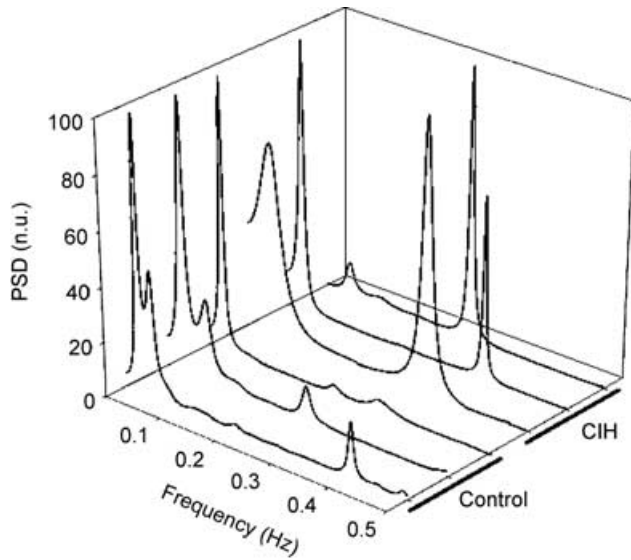
Figure 5. Effects of CIH on power spectral density of heart rate variability in three CIH and three control cats
PSD, power spectral density in normalized units (n.u).

there is also evidence for central neural alteration, amplifying the central integration of CB chemoafferent inputs following CIH (Mitchell *et al.* 2001). Indeed, long-term facilitation of ventilatory response is elicited by
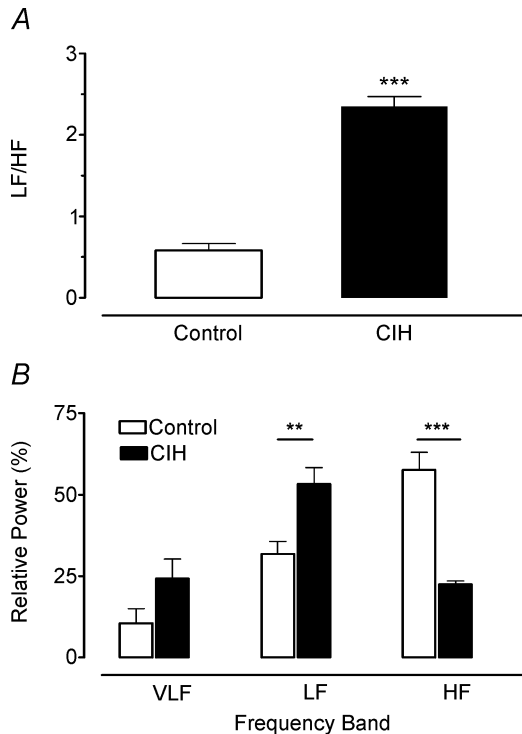

Figure 6. Effects of CIH on the low/high frequency ratio (*A*) and distribution of relative power spectral density (*B*) of very-low- (VLF), low- (LF) and high-frequency (HF) bands in six CIH cats
**P < 0.01 and ***P < 0.001, as compared with six control cats (Bonferroni test after two-way ANOVA). Error bars show S.E.
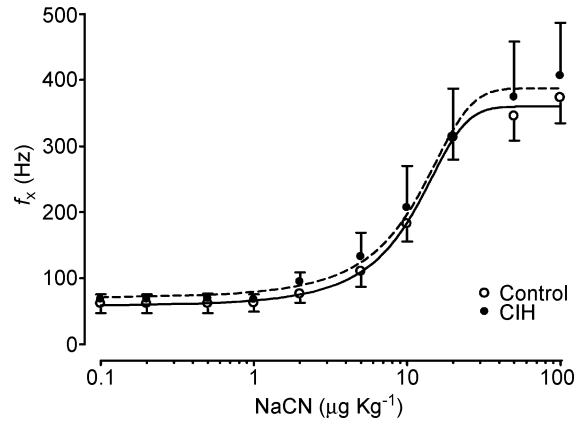

Figure 7. Dose–response curves for increases in frequency of chemosensory discharges (*f*$_x$) induced by NaCN in four CIH (●, dashed line) and six control cats (○, continuous line)
No significant statistical difference between curves (two-way ANOVA and Bonferroni test). Error bars show S.E.

intermittent but not sustained hypoxia, indicating that there are persistent changes in the output of the respiratory central pattern generator following intermittent peripheral chemoreceptor stimulation by hypoxia (Mitchell *et al.* 2001; Morris & Gozal, 2004).

Chronic intermittent hypoxia has been associated with augmented HVR in OSA patients and in animal models. Most of the evidence suggesting that HVR is augmented comes from studies performed in OSA patients (Cistulli & Sullivan, 1994; Narkiewicz *et al.* 1999; Mateika & Ellythy, 2003). However, in some studies a normal or even attenuated HVR has been also observed in patients with a long-term OSA history (Costes *et al.* 1995; Osanai *et al.* 1999). Nevertheless, a main problem with these
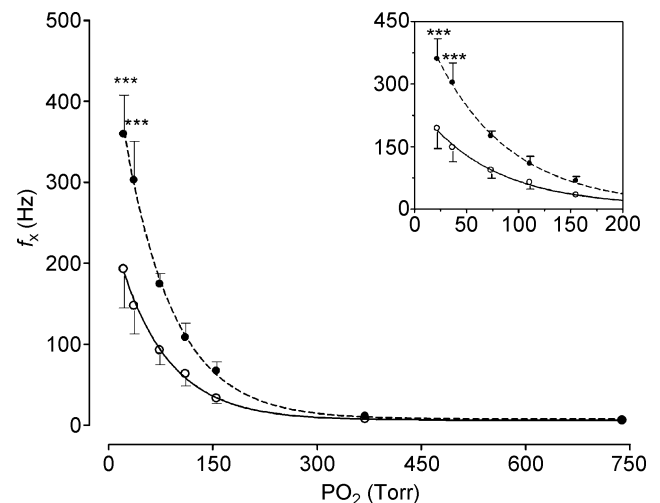

Figure 8. Frequency of chemosensory discharges (*f*$_x$) recorded at several levels of *P*$_{O_2}$ in four CIH (●, dashed line) and six control cats (○, continuous line)
***P < 0.001 (Bonferroni test after two-way ANOVA). Inset: detail of the same data, which illustrates the response in the hypoxic range.

studies is that peripheral chemoreflexes may be impaired by comorbidities such as obesity, ageing, hypertension and application of continuous positive airway pressure to treat the apnoeas. In a controlled study, done in patients with recently diagnosed OSA without treatment, Narkiewicz *et al.* (1999) found that reflex ventilatory, sympathetic and cardiovascular responses to acute hypoxia were enhanced. Studies performed in animal models have shown different effects of CIH on HVR. Ling *et al.* (2001) reported that phrenic HVR is enhanced in rats exposed to 5 min of ~10% O_2, followed by 5 min of air, 12 h $night^{-1}$ for seven nights. Similarly, Reeves *et al.* (2003) found that rats exposed to a CIH pattern consisting of alternating episodes of room air and ~10% O_2 for 90 s for 30 days, showed an enhanced HVR. However, Greenberg *et al.* (1999) did not find any differences in ventilatory responses to hypoxia or hypercapnia in rats exposed for 30 s to 6.5–7% O_2 followed by 30 s of normoxia, for 8 h day^{-1} for 10 days. Thus, the mechanism by which CIH alters the HVR seems to be highly dependent on the pattern and severity of CIH.

Effects of CIH on cardiovascular response and heart rate variability

Chronic exposure to intermittent hypoxia produces sustained systemic hypertension in rats (Greenberg *et al.* 1999; Fletcher, 2000). The hypertension has been attributed to an increased sympathetic outflow due to the repetitive hypoxic CB stimulation. Indeed, the denervation of the CB eliminates the hypertension induced by CIH (Fletcher *et al.* 1992; Bao *et al.* 1997). Fletcher *et al.* (1992) found an elevated arterial pressure in rats after 35 days of CIH exposure, but not after 10 days. On the contrary, Sica *et al.* (2000) and Peng *et al.* (2003) found that systolic arterial pressure increases in rats after 7 days of CIH with a pattern similar to the one employed by Fletcher *et al.* (1992, 1995). Narkiewicz *et al.* (1998*b*) found an augmented HVR in patients recently diagnosed with OSA. These patients were normotensive, without history of medication, and their blood gases were comparable to those of control subjects. Cistulli & Sullivan (1994) also found a similar increase of hypoxic ventilatory response in patients with severe OSA. Thus, the present results suggest that the time required for increasing arterial pressure and heart rate is longer than the time required to enhance HVR. Four days of intermittent hypoxia seem to be of insufficient duration to increase basal arterial pressure or cardiovascular responses to hypoxia. However, the spectral analysis of the RR interval data indicates that autonomic modulation of heart rate is early modified by CIH. We found that the LF/HF ratio was significantly higher ($P < 0.001$) in CIH cats (2.4 ± 0.1) as compared to control cats (0.6 ± 0.1). The spectral analysis of RR interval data indicates a clear effect of CIH that resembles what is observed in OSA patients. Certainly, patients with

long-term or recently diagnosed OSA have an increased LF/HF ratio, and a relative predominance of the LF component of heart rate variability, with a reduced HF component (Shiomi *et al.* 1996; Narkiewicz *et al.* 1998*a*). In our model, we observed that CIH increases the LF component with a simultaneous reduction of the HF component. Thus, cats exposed to CIH in this study, similarly to OSA patients, show an increased LF/HF ratio suggesting the existence of early changes in autonomic control of heart rate. Nevertheless, we have to emphasize that these changes appear in cats after 4 days of exposure to CIH, compared to OSA patients, who usually have a longer history of snoring and disordered breathing during sleep. Thus, LF/HF ratio could be used as an early index of autonomic changes induced by CIH. Although it is still under debate, it is widely accepted that the LF/HF ratio is a measure of autonomic influences on heart rate variability (Task Force, 1996; Otzenberger *et al.* 1998). The LF and HF components are related to autonomic influences on heart rate (Task Force, 1996); while the HF component has been related to cardiac parasympathetic efferent activity, some authors consider that the LF interval of spectral power density is modulated by sympathetic cardiac efferences (Task Force, 1996; Pagani *et al.* 1997).

In summary, the present study shows that the CIH pattern of short episodes of hypoxia selectively enhances cat CB chemosensory and ventilatory responses to hypoxia after few days. Cardiovascular responses to hypoxia were not enhanced by CIH; however, the analysis of RR interval data showed a marked modification of the power spectral density distribution and an increased LF/HF ratio, similar to what is observed in OSA patients.

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Acknowledgements