Chapter 42
Rabbit Ventilatory Responses to Peripheral Chemoexcitators: Effects of Chronic Hypoxia

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Abstract Exposure to prolonged hypobaric hypoxia increases baseline ventilation and the ventilatory response to acute hypoxia, a phenomenon known as hypoxic ventilatory acclimatization (HVA). It is currently accepted that the carotid bodies and the reduced PO_2 levels are key elements in the generation of the HVA. However, because most of these experiments have been performed in hypobaric conditions, we studied the effects of 15 days of chronic normobaric hypoxia (CNH) on the rabbit ventilatory responses to hypoxia and chemoexcitatory molecules. New Zealand White rabbits were placed in a 0.3 m³ chamber with controlled temperature and a mean O_2 content of 9.17 ± 0.09 %. Animals with or without CNH exposition (naïve) were anesthetized (ketamine/xylazine 75/7.5 mg/kg, i.m.), cannulated and air flow was measured. In naïve animals hypoxic challenges and NaCN increase ventilation, effect completely abolished after bilateral chemodenervation. However, ventilatory responses to nicotine, ATP and dopamine remained largely unchanged after bilateral chemodenervation, suggesting a centrally mediated effect for these drugs. Basal ventilation was reduced in CNH animals, but the dose dependent ventilatory increases induced by NaCN presented an increased sensibility. Further experiments are needed to elucidate the mechanisms responsible for these acclimatized responses.

Keywords Chronic hypoxia • Ventilatory acclimatization • Ventilatory reflexes • Acetylcholine • ATP • Dopamine • Cyanide •

42.1 Introduction

Exposure to high altitude augments the ventilatory response to acute hypoxia in humans and animals, a phenomenon known as ventilatory acclimatization (Bisgard 2000). The hypoxic ventilatory acclimatization (HVA) is a key physiological response in animals subjected to prolonged hypobaric

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hypoxia. Ventilation increases progressively and continues increasing despite the maintenance of the same hypoxic level and finally the baseline ventilation is reset to a higher level than the pre hypobaric level. This HVA is accompanied by an increased ventilatory response to acute hypoxia, indicating an increased ventilatory sensibility and/or reactivity. It is currently accepted that the carotid body (CB) (Bisgard 2000; Powell et al. 2000a) and the reduced PO_2 levels (Bisgard et al. 1986a, b) are key components in the generation of the HVA. However, many of these experiments have been performed under hypobaric conditions (Aaron and Powell 1993; Dwinell and Powell 1999) assuming that O_2 *per se* is the only important variable in producing the HVA, despite of the fact that barometric pressure is substantially reduced. Thus, to separate the effects of hypoxia from the reduced barometric pressure, we studied the effects of chronic normobaric hypoxia (CNH) on basal ventilation and the ventilatory responses elicited by acute hypoxia in the rabbit, and the effects of several chemoexcitatory molecules.

42.2 Methods

42.2.1 Normobaric Hypoxic Exposure

Animals subjected to chronic normobaric hypoxia (CNH) were weighed and their hematocrit measured from blood withdrawn from an ear vein before they were placed in a 0.3 m^3 (0.6 × 0.5 × 1.0 m) acrylic chamber. The chamber was initially purged with pure N_2 until the F_IO_2 reached ~ 8.5%. The F_IO_2 was continuously monitored in the chamber with an O_2 sensor (AX300, Teledyne Analytical Instruments, USA) connected to an electronic controller (Zelio SR2 B121BD, Schneider Electric, France), which admitted air or N_2 to maintain F_IO_2 between 8.42% and 10.24%, with a mean level of 9.17 ± 0.09% (mean ± SD; 5 h periods in 10 different experiments). The animals were maintained in this hypoxic environment for 15 days. Every 48 h the chamber was opened for a 5–10 min period necessary for cleaning and restitution of water and food. The ventilatory water and CO_2, and urine volatiles were trapped with a desiccant, $CaCO_3$ and boric acid, respectively. At the end of the CNH period the animals were weighed again and their hematocrit measured.

42.2.2 Physiological Recordings

Ventilatory and cardiovascular variables were recorded from male New Zealand White rabbits (1.77 ± 0.07 kg) without (naïve) or with exposure to CNH. The animals were initially anesthetized (ketamine/xylazine 75/7.5 mg/kg, i.m.) and intubated to measure air flow with a pneumotacograph. The right saphenous vein, the lingual artery and the left femoral artery were catheterized to maintain a surgical level of anesthesia (sodium pentobarbitone, 24 mg/h), apply drugs to the CB and measure arterial blood pressure (Pa), respectively. Tidal volume (V_T), minute volume (V_I) and ventilatory frequency (F_V) were derived from the air flow signal. Cardiac frequency (F_C) was assessed from the electrocardiographic signal recorded in the first derivative. All physiological signals were digitally recorded (PowerLab, ADInstruments) for later analysis. Intracarotid injections of nicotine (0.1–200 μg/kg), ATP (0.1–500 μg/kg), dopamine (0.1–200 μg/kg) and NaCN (0.1–100 μg/kg), and different F_IO_2 levels (0–100%) were used to evaluate the peripheral chemoreflexes. The carotid nerves were severed bilaterally and the vagus nerves cut central to the nodose ganglion.

At the end of the recording session the animals were sacrificed by an anesthetic overdose. Bio-Ethics Committees from the Facultad de Ciencias, Universidad de Chile, and Facultad de Ciencias Biológicas,

P. Universidad Católica de Chile approved the experimental protocols. All data expressed as mean ± SEM, excepted when stated. Statistical analyses performed according to data structure, and decision criteria set at $P<0.05$.

42.3 Results

42.3.1 Responses of Naïve Rabbits to Chemoreceptor Stimulation

Acute hypoxic challenges produced a dose dependent increase in V_I, while hyperoxia reduced it. The changes in V_I were produced by changes in V_T and F_V, although percent changes in volume were larger than dose of frequency in all experimental F_IO_2 values tested. Arterial pressure and heart rate were slightly reduced during hypoxic challenges. All these responses were completely abolished after bilateral section of the carotid and aortic nerves.

Nicotine produced a slight increase in V_I, mostly due to increases in V_T and large but variable changes in F_V. These changes were accompanied by a dose-dependent decrease in both Pa and F_C. Total peripheral chemodenervation had no significant effect on the ventilatory response induced by the maximal nicotine dose (Fig. 42.1a). Intracarotid applications of ATP reduced Pa and F_C in

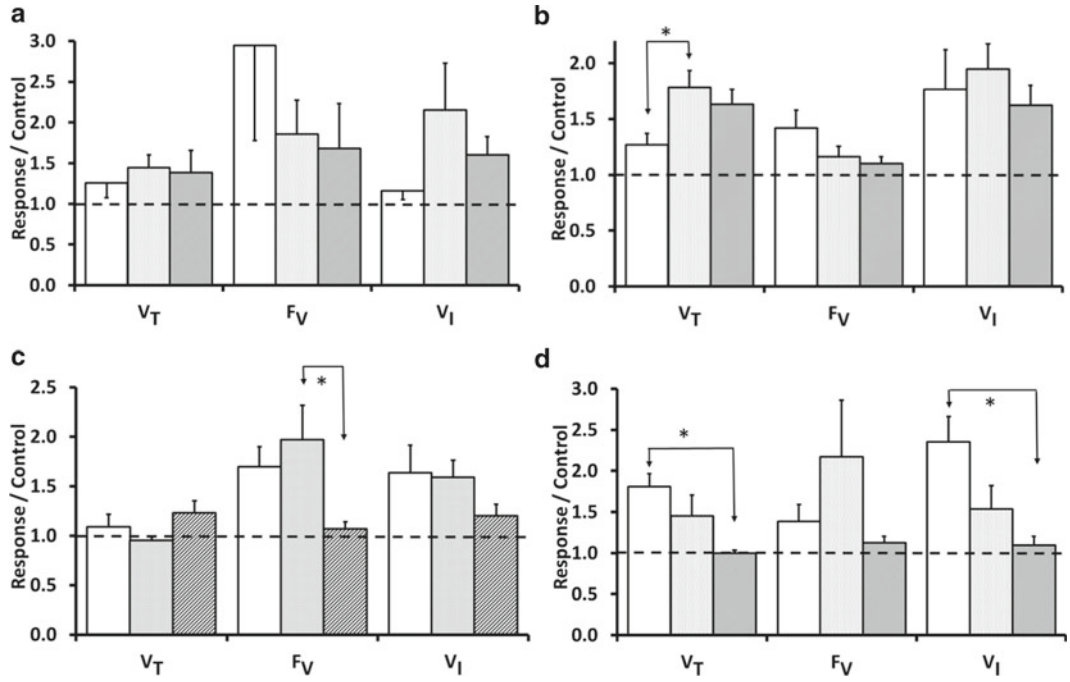

Fig. 42.1 Ventilatory effects of peripheral chemoreceptors chemoexcitants in control (empty bars) and after successive bilateral carotid (*light gray bars*) and aortic (*dark gray bars*) nerve section. (**a)** Responses elicited by nicotine maximal doses (100 μg/kg) were not significantly modified by denervation. (**b**) Responses induced by maximal ATP doses (100 μg/kg) were not significantly modified by denervation, except for a significant increase in V_T after carotid denervation that was reverted by total chemodenervation. (**c**) Responses induced by maximal DA doses (100 μg/kg) were not significantly modified by denervation, except for a significant decrease in F_V after total chemodenervation. (d) Responses induced by maximal NaCN doses (200 μg/kg) were abolished by total chemodenervation. *$P<0.05$ repeated measures ANOVA. $N=5$

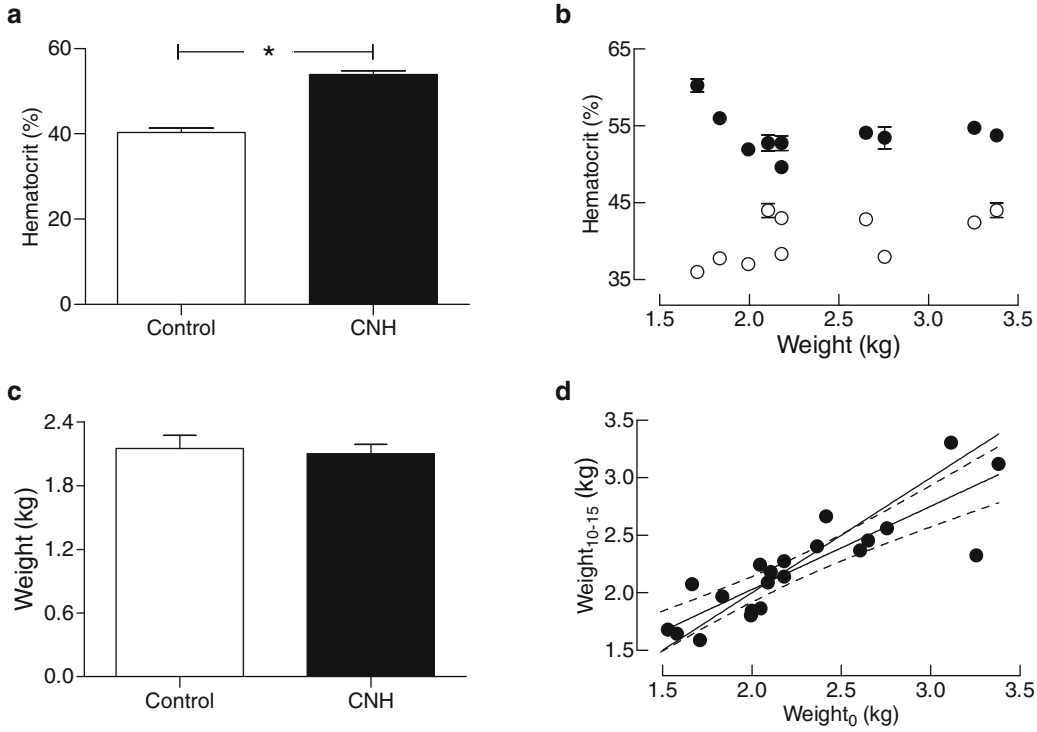

Fig. 42.2 Changes in hematocrit and weight induced by 15 days of chronic normobaric hypoxia (CNH). (**a**) Rabbits subjected to NCH (*filled bar*) significantly (*, $P<0.0001$; paired *t*-test; $n=10$) increased their hematocrit with respect to their initial values (*empty bar*). (**b**) The initial (empty circles) and final (*filled*) hematocrit were uncorrelated ($P>0.05$; F-test; $n=10$) with weight. (**c**) The animals gain no weight ($P>0.05$; Wilcoxon paired ranks test) during the CNH period. (**d**) The weight at the end of the CNH period was related to the initial weight ($P<0.001$; F-test; $n=19$) with a slope significantly lower than 1 ($P<0.001$; F-test)

a dose-dependent manner and slightly increased V_I by increasing both V_T and F_V. Denervation of carotid bodies slightly and significantly increased V_T responses induced by maximal ATP doses (Fig. 42.1b, $P<0.05$, repeated measures ANOVA), but ventilatory responses to maximal ATP doses remained largely unmodified after total peripheral chemodenervation (Fig. 42.1b). Dopamine increased V_I in a dose dependent manner, largely due to changes in F_V without major modifications in V_T. These changes were accompanied by reductions in Pa that were not accompanied by modifications of F_C. Bilateral total chemodenervation had no major impact on evoked ventilatory responses, except for a non-significant reduction of F_V with respect to controls but significant ($P<0.05$, repeated measures ANOVA) with respect to carotid denervation (Fig. 42.1c). NaCN produced a slight reduction of Pa and F_C and an increase in V_I, due to increases of V_T and F_V. Bilateral carotid chemodenervation reduced slightly but not significantly both V_T and V_I, while total peripheral chemodenervation significantly reduced V_T and V_I ($P<0.05$, repeated measures ANOVA), without significantly modifying F_V (Fig. 42.1d).

42.3.2 Physiological Modifications in Rabbits Subjected to CNH

After 15 days of CNH the hematocrit (Fig. 42.2a) of the animals increased significantly from $40.34\pm1.01\%$ to $53.94\pm0.89\%$ ($P<0.05$; Student paired *t*-test; $n=10$). The animals did not gain

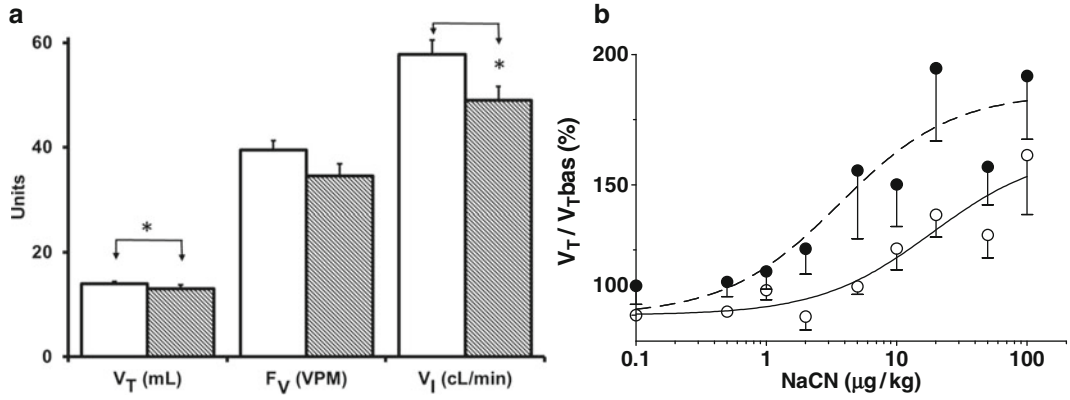

Fig. 42.3 Effects of CNH on rabbit basal ventilation in normoxia and responses to NaCN. (**a**) Basal (normoxic) V_T and V_I were significantly reduced (* $P<0.05$; Student *t*-test) in CNH (*lined bars*; n = 5) with respect to naïve (*empty bars*; n = 11) rabbits, without modification of F_V. (**b**) Dose response changes in V_T induced by NaCN in naïve (*empty dots*, *continuous line*; n = 5) and in CNH (*filled dots*, *segmented line*; n = 7) animals. ED_{50} was significantly reduced from 18.6 ± 10.4 to 3.6 ± 2.3 ($P<0.01$; 2 way ANOVA) in CNH rabbits

weight during the CNH exposure (Fig. 42.2c). We found a significant linear correlation between the initial and final weight, although the slope was significantly lower than unity (Fig. 42.2d). There was no significant correlation between the weight and the hematocrit at the beginning or the end of the CNH period (Fig. 42.2b), or between the initial and final hematocrit.

Basal V_I and V_T were significantly reduced ($P<0.05$; Student *t*-test) in CNH animals with respect to naïve ones, while F_V was reduced without reaching significance level (Fig. 42.3a).

42.3.3 Responses of CNH Rabbits to Chemoreceptor Stimulation

Intracarotid injections of NaCN produced a dose dependent increase in V_T and V_I that was dependent on both carotid and aortic chemosensory innervation as in naïve rabbits (Fig. 42.1d). However, the dose–response curve of CNH rabbits was significantly shifted to the left with respect to naïve animals. Thus, ED_{50} was reduced from 18.6 ± 10.4 µg/kg in naïve animals to 3.6 ± 2.3 µg/kg in CNH animals ($P<0.01$; 2 way ANOVA), without significant modification of the slope or maximal response ($P>0.01$; 2 way ANOVA).

42.4 Discussion

The ventilatory and cardiovascular responses to acute hypoxia (Chalmers et al. 1967; Korner and Edwards 1960) and NaCN (Docherty and McQueen 1979; Matsumoto 1986) were similar to those previously reported. However, CB denervation reduced the responses that were abolished only after total peripheral chemodenervation. In non-anesthetized rabbits, the hypoxic ventilatory responses are completely dependent on carotid afferents with negligible participation of the aortic chemoreceptors (Bouverot et al. 1973; Chalmers et al. 1967; Korner and Edwards 1960). The observed differences could be partly explained by the use of anesthetic.

In naïve rabbits, nicotine and dopamine increased V_I, increasing V_T and F_V the former and mostly F_V the latter. Carotid denervation had no significant effect on the ventilatory responses induced by

maximal doses of nicotine or dopamine, while total peripheral chemodenervation only significantly reduced F_V responses induced by maximal dopamine doses. Nicotine increases (Docherty and McQueen 1979) while dopamine reduces chemosensory discharges (Docherty and McQueen 1979; Ponte and Sadler 1989). ATP increased V_T, F_V and V_I, and reduced Pa and F_R, effects that were not abolished by total peripheral chemodenervation. It noteworthy that carotid denervation significantly augmented F_V increases induced by ATP. Thus, the increases in ventilation elicited by maximal nicotine, dopamine and ATP doses which were resistant to peripheral chemodenervation may result from centrally mediated effects of these drugs.

The 15 day CNH period produced a significant increase of the rabbit hematocrits. The initial hematocrit was similar to that previously reported in neonatal (Baker et al. 1997) and adult rabbits (Harcourt-Brown and Baker 2001), and the final levels were similar to those obtained after 30 days of CNH in rabbits subjected to similar F_IO_2 (Baker et al. 1997). On the other hand, the rabbits show no increase in weight during the CNH exposure, similar to neonatal rabbits subjected to a similar F_IO_2 level for 30 days (Baker et al. 1997). Similar changes have been reported in cats subjected to CNH for 28 days (Barnard et al. 1987). Thus, the observed changes in these variables indicate that the hypoxic challenge was sufficient to evoke a physiologically adaptive response similar to that previously reported in rabbits and cats.

In CNH rabbits V_T and V_I were significantly reduced with respect to naïve animals, without a significant reduction in F_V. Thus, opposed to other animal or chronic hypobaric hypoxia models (Aaron and Powell 1993; Chen et al. 2002) basal ventilation appeared depressed in CNH rabbits, suggesting that other components of the ventilatory regulatory pathway are modulated in a different way in this species. Basal ventilation increases in cats subjected to CNH for 28 days, with a concomitant increase in CB chemosensory afferent responses to acute hypoxia (Barnard et al. 1987). However, CB chemosensory afferent activity at normoxic and hyperoxic levels was not significantly modified by CNH (Barnard et al. 1987). Thus, normoxic afferent chemosensory activity could remain unchanged in CNH rabbits, and the changes in basal ventilation may result from modifications at different levels of the ventilatory control (Powell et al. 2000b). Conversely, discharges recorded from *in vitro* isolated CB preparations from chronic hypobaric hypoxic animals show a time dependent increase of discharge at normoxia and hyperoxia that reaches a plateau after about 9 days of treatment (Chen et al. 2002). Altogether, these data suggest that chemosensory hypoxic sensitivity could be differentially modulated by PO_2 and the atmospheric pressure. Recording the afferent chemosensory activity in CNH rabbits could provide further insight on the mechanisms involved in the CNH reduced normoxic ventilation.

Because most of the ventilatory effects induced by the chemoexcitants tried in this study in naïve animals appear to exert their effects through central mechanisms, we evaluated the ventilatory reflexes in CNH rabbits only with intracarotid NaCN injections. The relationship between the elicited ventilatory responses and the NaCN dose in CNH rabbits presented similar maximal increases and a similar slope than in naïve animals, suggesting that similar mechanisms operate in both CNH and naïve animals. However, the ED_{50} of the curve was reduced in CNH with respect to naïve animals, suggesting an increased sensitivity in the ventilatory response. Chemosensory denervation completely abolished this NaCN-induced response indicating that chemosensory reflex sensitivity is increased in CNH, and suggests that this increase is at the peripheral chemoreceptor level. Changes in transmitter and receptor expression (Chen et al. 2002) or transmitter relevance (He et al. 2005) have been reported to occur in the rat CB as a result of chronic hypoxia. Moreover, electrical properties of CB chemoreceptor cells are also modified in chronic hypoxic cell cultures (Kääb et al. 2005; Stea et al. 1995). Thus, several changes at the transduction and/or transmission processes in the CB can be modified by CNH leading to an increase in the peripheral chemoreceptor sensitivity to NaCN stimulation.

In summary, in naïve rabbits ventilatory responses to hypoxia and NaCN are dependent on peripheral chemosensory inputs, while nicotine, dopamine and ATP exert their effects through other peripheral afferents or central mechanisms. Rabbits subjected to CNH present reduced ventilation, but their responses to NaCN are increased in sensitivity. Further experiments are needed to elucidate the mechanisms responsible for these acclimatized responses.

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References

Aaron EA, Powell FL (1993) Effect of chronic hypoxia on hypoxic ventilatory response in awake rats. J Appl Physiol 74:1635–1640

Baker JE, Curry BD, Olinger GN, Gross GJ (1997) Increased tolerance of the chronically hypoxic immature heart to ischemia Contribution of the KATP channel. Circulation 95:1278–1285

Barnard P, Andronikou S, Pokorski M, Smatresk N, Mokashi A, Lahiri S (1987) Time-dependent effect of hypoxia on carotid body chemosensory function. J Appl Physiol 63:685–691

Bisgard GE (2000) Carotid body mechanisms in acclimatization to hypoxia. Respir Physiol 121:237–246

Bisgard GE, Busch MA, Forster HV (1986a) Ventilatory acclimatization to hypoxia is not dependent on cerebral hypocapnic alkalosis. J Appl Physiol 60:1011–1015

Bisgard GE, Busch MA, Daristotle L, Berssenbrugge AD, Forster HV (1986b) Carotid body hypercapnia does not elicit ventilatory acclimatization in goats. Respir Physiol 65:113–125

Bouverot P, Candas V, Libert JP (1973) Role of the arterial chemoreceptors in ventilatory adaptation to hypoxia of awake dogs and rabbits. Resp Physiol 17:209–219

Chalmers JP, Korner PI, White SW (1967) The relative roles of the aortic and carotid sinus nerves in the rabbit in the control of respiration and circulation during arterial hypoxia and hypercapnia. J Physiol 188:435–450

Chen J, He L, Dinger B, Stensaas L, Fidone S (2002) Role of endothelin and endothelin a-type receptor in adaptation of the carotid body to chronic hypoxia. Am J Physiol Lung Cell Mol Physiol 282:L1314–L1324

Docherty RJ, McQueen DS (1979) The effects of acetylcholine and dopamine on carotid chemosensory activity in the rabbit. J Physiol 288:411–423

Dwinell MR, Powell FL (1999) Chronic hypoxia enhances the phrenic nerve response to arterial chemoreceptor stimulation in anesthetized rats. J Appl Physiol 87:817–823

Harcourt-Brown FM, Baker SJ (2001) Parathyroid hormone, haematological and biochemical parameters in relation to dental disease and husbandry in rabbits. J Small Anim Pract 42:130–136

He L, Dinger B, Fidone S (2005) Effect of chronic hypoxia on cholinergic chemotransmission in rat carotid body. J Appl Physiol 98:614–619

Kääb S, Migel-Velado E, López-López JR, Pérez-García MT (2005) Down regulation of Kv3.4 Channels by chronic hypoxia increases acute oxygen sensitivity in rabbit carotid body. J Physiol Lond 566:395–408

Korner PI, Edwards AWT (1960) The immediate effects of acute hypoxia on the heart rate, arterial pressure, cardiac output and ventilation of the unanaesthetized rabbit. Exp Physiol 45:113–122

Matsumoto S (1986) Effects of carotid body chemoreceptor stimulating and depressing agents on internal intercostal muscle activity in the rabbit. Jap J Physiol 36:1001–1013

Ponte J, Sadler CL (1989) Interactions between hypoxia, acetylcholine and dopamine in the carotid body of rabbit and cat. J Physiol 410:395–410

Powell FL, Dwinell MR, Aaron EA (2000a) Measuring ventilatory acclimatization to hypoxia: comparative aspects. Respir Physiol 122:271–284

Powell FL, Huey KA, Dwinell MR (2000b) Central nervous system mechanisms of ventilatory acclimatization to hypoxia. Respir Physiol 121:223–236

Stea A, Jackson A, Macintyre L, Nurse CA (1995) Long-term modulation of inward currents in O_2 chemoreceptors by chronic hypoxia and cyclic AMP *in vitro*. J Neurosci 15:2192–2202