12

Acute Effects of Systemic Erythropoietin Injections on Carotid Body Chemosensory Activity Following Hypoxic and Hypercapnic Stimulation

David C. Andrade, Rodrigo Iturriaga, Florine Jeton, Julio Alcayaga, Nicolas Voituron, and Rodrigo Del Rio

Abstract

The carotid body (CB) chemoreceptors sense changes in arterial blood gases. Upon stimulation CB chemoreceptors cells release one or more transmitters to excite sensory nerve fibers of the carotid sinus nerve. While several neurotransmitters have been described to contribute to the CB chemosensory process less is known about modulatory molecules. Recent data suggest that erythropoietin (Epo) is involved in the control of ventilation, and it has been shown that Epo receptor is constitutively expressed in the CB chemoreceptors, suggesting a possible role for Epo in regulation of CB function. Therefore, in the present study we aimed to determine whether exogenous applications of Epo modulate the hypoxic and hypercapnic CB chemosensory responses. Carotid sinus nerve discharge was recorded in-situ from anesthetized adult male and female Sprague Dawley rats (350 g, n = 8) before and after systemic administration of Epo (2000 UI/kg). CB-chemosensitivity to hypoxia and hypercapnia was calculated by exposing the rat to F_iO_2 5–15% and F_iCO_2 10% gas mixtures, respectively. During baseline recordings at normoxia, we found no effects of Epo on CB activity both in male and

Senior authors Nicolas Voituron and Rodrigo Del Rio have equally contributed to this chapter.

D. C. Andrade
Laboratory of Cardiorespiratory Control, Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile

R. Iturriaga
Laboratorio de Neurobiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

F. Jeton · N. Voituron
Laboratoire Hypoxie & Poumon, Universite Paris 13, Paris, France

J. Alcayaga
Laboratorio de Fisiología Celular, Universidad de Chile, Santiago, Chile

R. Del Rio (✉)
Laboratory of Cardiorespiratory Control, Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile

Centro de Envejecimiento y Regeneración (CARE), Pontificia Universidad Católica de Chile, Santiago, Chile

Centro de Excelencia en Biomedicina de Magallanes (CEBIMA), Universidad de Magallanes, Punta Arenas, Chile
e-mail: rdelrio@bio.puc.cl

E. B. Gauda et al. (eds.), *Arterial Chemoreceptors*, Advances in Experimental Medicine and Biology 1071, https://doi.org/10.1007/978-3-319-91137-3_12

female rats. In addition, Epo had no effect on maximal CB response to hypoxia in both male and female rats. Epo injections enhanced the maximum CB chemosensory response to hypercapnia in female rats (before vs. after Epo, 72.5 ± 7.1 Hz vs. 108.3 ± 6.9 Hz, $p < 0.05$). In contrast, Epo had no effect on maximum CB chemosensory response to hypercapnia in male rats but significantly increased the response recovery times (time required to return to baseline discharge following hypercapnic stimulus) from 2.1 ± 0.1 s to 8.2 ± 2.3 s ($p < 0.05$). Taken together, our results suggest that Epo has some modulatory effect on the CB chemosensory response to hypercapnia.

Keywords
Carotid Body · Carotid sinus nerve · Erythropoietin · Hypoxia · Hypercapnia

12.1 Introduction

Regulation of breathing by arterial blood gases depend on peripheral and central chemoreceptors (Feldman et al. 2003; Guyenet 2014). The carotid Body (CB), the main peripheral chemoreceptor, is sensitive to changes in arterial blood gas composition (PO_2, PCO_2 and pH) (Gonzalez et al. 1992), initiating a ventilatory and sympathetic responses to maintain cardio-respiratory homeostasis (Gonzalez et al. 1995; Despas et al. 2006; Lopez-Barneo et al. 2016). Furthermore, recent studies indicate that peripheral chemoreceptors determine the sensitivity of the central chemoreceptors to CO_2 (Smith et al. 2015). Chemoreflex responses serve to maintain PCO_2 and pH within homeostatic parameters in the internal medium and to adjust the O_2 supply according to the metabolic demand (Gonzalez et al. 1992). Upon stimulation, the CB chemoreceptor cells release neurotransmitters that activate the sensory nerve fibers of the carotid sinus nerve (CSN) which relay this information to brainstem neurons that regulate breathing (Prabhakar and Peers 2014). In addition, the CB is sensitive to plasma levels of Erythropoietin (Epo) (Soliz et al. 2005). Previous studies suggest that CB chemotransduction is modulated by high Epo plasma levels (Soliz et al. 2007). EPO's role in synthesis of red blood cells is well known (Jelkmann 1992, 2011). However Epo is also produced by neurons and glial cells (Digicaylioglu et al. 1995). Furthermore, Epo-receptors (EpoR) are widely distributed in the areas known to be associated with respiratory control (Soliz et al. 2005) such as the Respiratory Rhythm Generator, the CBs and central chemoreceptor sensitive areas (i.e. nucleus of the solitarii tract and raphe). Interestingly, expression of Epo and its receptor has been observed in CB glomus cells (Lam et al. 2009). The presence of EpoR in these structures supports the notion that Epo participates in respiratory regulation and CB chemotransduction. Indeed, previous work indicates that Epo may regulate the hypoxic ventilatory response (HVR) in humans and mice in a sex-dependent manner (Jeton et al. 2017; Pichon et al. 2016; Soliz 2013; Soliz et al. 2012; Voituron et al. 2014). Thus, it is possible that Epo acting on EpoR located within the CB tissue may play a role in the regulation of the HVR. However, there is no direct evidence for the role played by Epo on CB chemosensory function.

Our goal was to assess whether exogenous applications of Epo could modulate the hypoxic and hypercapnic CB chemosensory responses. To assess this, carotid sinus nerve discharge was recorded *in-situ* from anesthetized adult male and female Sprague Dawley rats before and after systemic administration of Epo.

12.2 Methods

12.2.1 Ethical Approval

Experiments were performed in adult male (n = 4) and female (n = 4) Sprague Dawley rats (~350g). All experiments were approved by the Bioethical Committee of the P. Universidad Católica de Chile and were carried out under the guidelines of the American Physiological Society,

the Guía para el Cuidado y Uso de los Animales de Laboratorio from CONICYT and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

12.2.2 Animal and Procedures

After deep anesthesia with sodium pentobarbital (40 mg/kg I.P.), rats (n = 8) were placed in supine position and the rectal temperature was maintained at 38.0 ± 0.5 °C with a regulated heating pad. Carotid sinus nerve discharge was recorded *in-situ* as previously described (Del Rio et al. 2010, 2011). Briefly, right carotid sinus nerve was isolated and placed on a pair of platinum electrodes and covered with warm mineral oil. Signal was then amplified (Grass P511, USA), filtered (30–500 Hz) and sent to an electronic spike-amplitude discriminator to select action potentials of given amplitude above the noise. Hyperoxic test (100% O_2) was used to set the threshold of CB sensory activity present in the electrophysiological recording. The selected action potentials were used to evaluate the CB chemosensory frequency of discharge (f_x expressed in Hz). Signals were acquired with an analog-digital system PowerLAB and analyzed with the Chart 7-Pro software (ADInstruments, Australia). CB-chemosensory sensitivity to hypoxia or hypercapnia was calculated by allowing the rat to spontaneously breathe F_iO_2 5–15% and F_iCO_2 3–7% gas mixtures, respectively, before and after 60 min of systemic administration of Epo (2000 UI/kg, 100 µl i.v. bolus).

12.2.3 Data Analysis and Statistics

The CB chemosensory frequency discharges (f_x) response-curves were fitted to the inspiratory PO_2 according to the following exponential function (Berkenbosch et al. 1991, 1997).

$$f_x = G\exp\left(-DPO_2\right) + A$$

In which, G is the overall gain, D is oxygen threshold and A is the value of CB chemosensory discharge measured during hyperoxic challenge (Dejour test). For the CB chemosensory response to hypercapnia, linear regression analysis was used. Results were expressed as mean ± standard errors of the mean (SEM). Differences were tested by Bonferroni test following two-ways ANOVA or by using Student T test. All analyses were performed with Graph Pad – Prism software (Graph Pad software, La Jolla, CA, USA). Differences were considered significant when $p < 0.05$.

12.3 Results

12.3.1 CB Chemosensory Activity in Male and Female Rats

Chemosensory activity in normoxia and during acute hypoxia did not differ between adult male and female rats (Table 12.1). There is a trend to have higher maximal responses to severe hypoxia in male rats compared to females. Indeed, the CB responses to F_iO_2 5% expressed as a percent change compared to the values at normoxia were 516.7 ± 31.4% in males and 404.0 ± 32.1% in females. However, this difference was not statistically different between both groups.

12.3.2 Effects of Epo Injection on CB Activity

During baseline recordings at normoxia, we found no effects of Epo on CB activity both in male and female rats (Fig. 12.1). In addition, acute Epo injection showed no effects in maximal CB response to hypoxia neither in CB oxygen sensitivity in male or female rats (Fig. 12.2).

Table 12.1 Carotid body chemosensory baseline activity in normoxia and in response to hypoxia

	♂	♀
F_iO_2 21% *fx* (Hz)	70.1 ± 14.1	80.9 ± 13.4
F_iO_2 10% *fx* (Hz)	310.6 ± 33.1	295.0 ± 29.0
F_iO_2 5% *fx* (Hz)	362.0 ± 22.0	327.0 ± 26.3

Data are showed as mean ± SEM. *fx*: Carotid body chemosensory activity; F_iO_2: Fraction of inspired oxygen

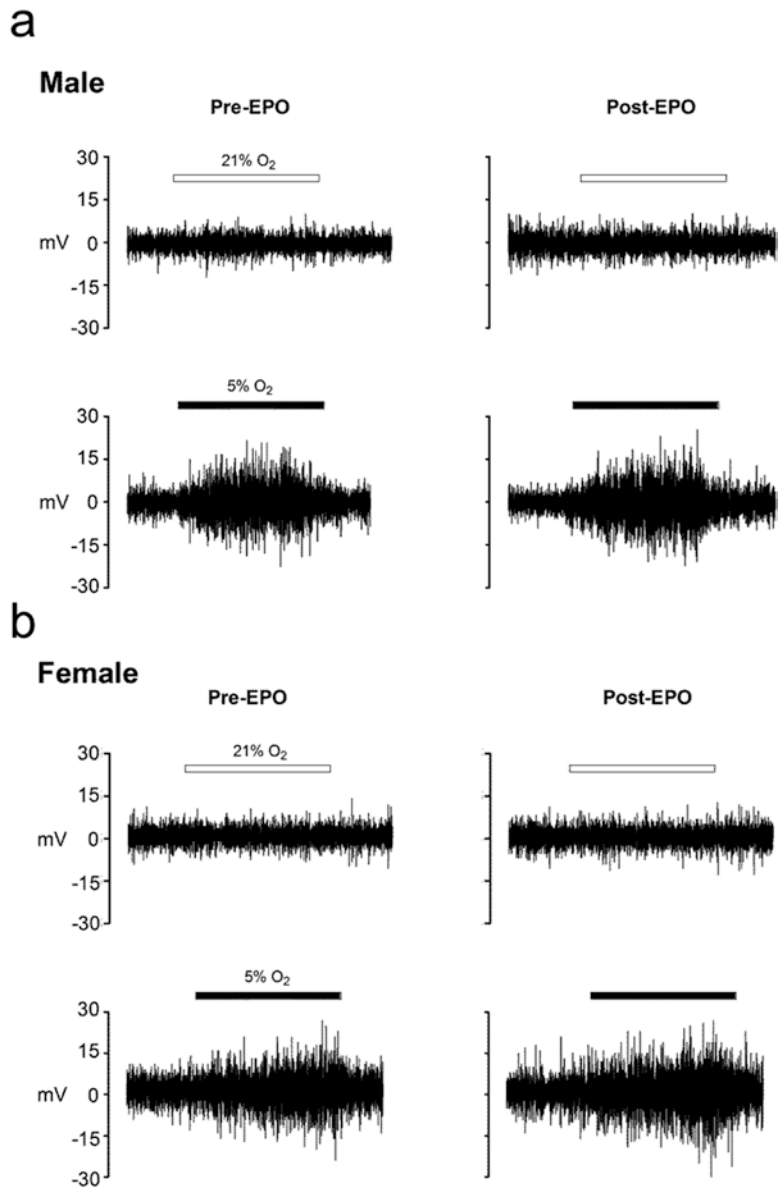

Fig. 12.1 Effects of Epo administration on carotid sinus nerve activity in male and female rats. (**a**), representative recordings of CB chemosensory activity *in situ* from male rats, during normoxic stimulation (Upper panel) before and after Epo i.v. injection. Note the absence of Epo effects on baseline CB activity under normoxia (Lower Panel), CB chemosensory activity during acute hypoxic (20s) stimulation before and after Epo administration. No effect of Epo on CB response to hypoxia was found. (**b**), representative CB recordings in female rats before and after Epo i.v. injection. Note the absence of Epo effects on baseline CB activity under normoxia

12.3.3 Effects of Epo on CB Chemosensory Response to Hypercapnia

The effect of Epo injections on CB activity is shown in Fig. 12.3. Interestingly, Epo administration increases the maximal CB chemosensory response to hypercapnia 10% CO_2 only in female rats (Fig. 12.4). Indeed, the female CB responses to hypercapnia were 72.5 ± 7.1 Hz vs. 108.3 ± 6.9 Hz ($P < 0.05$), before and after Epo, respectively. Contrarily, Epo has no effect on the maximal CB chemosensory response to hypercapnia in males but significantly increases the response recovery times (time required to return

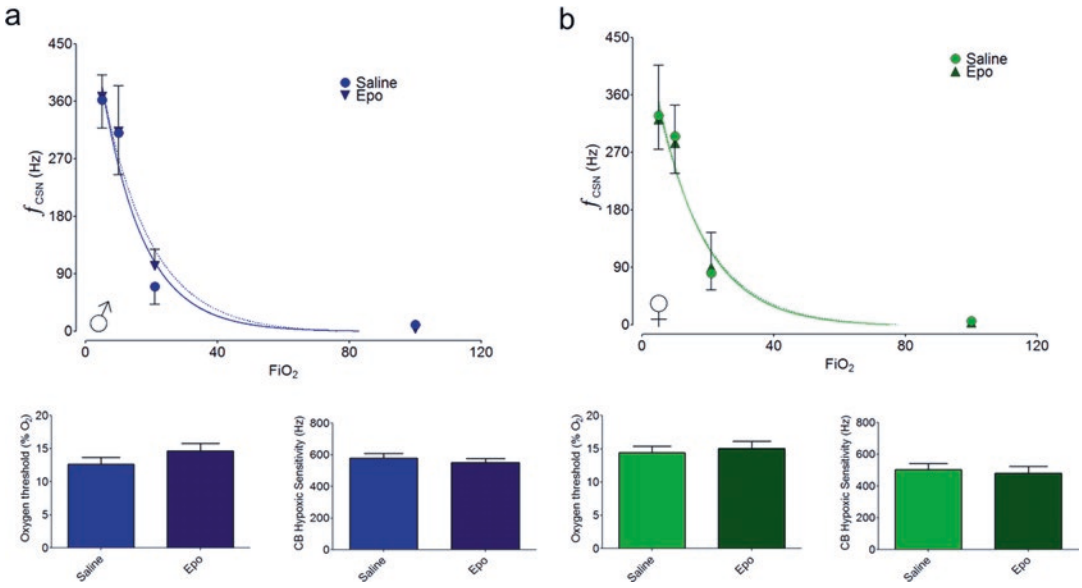

Fig. 12.2 Summary of the effects of Epo on carotid body chemosensory function in hypoxia. Upper panels, CB chemosensory response to hypoxia before and after Epo administration in (**a**) males and (**b**) females rats. P > 0.05, for CB chemosensory curves, Bonferroni test following two-ways ANOVA, n = 4 per group. Lower panels, Epo has no effect on oxygen threshold nor in CB hypoxic sensitivity in both males or females rats. P > 0.05, paired t test. n = 4 per group

to baseline discharge following hypercapnic stimulus) from 2.1 ± 0.1 s to 8.2 ± 2.3 s (Fig. 12.4).

12.4 Discussion

The main findings of our study were: (i) during normoxic condition Epo injection did not modify the CB chemosensory discharge in male and female rats; (ii) Epo injection did not change the CB chemosensory responses induced by hypoxia (10–5% O_2) neither in males nor in female rats; (iii) Epo injections enhanced the maximal CB chemosensory response to hypercapnia (10% CO_2) in female rats, and (iv) Epo injections increased the time needed to return to baseline discharge levels following hypercapnic stimulation in male rats. Together these results suggest that Epo modulate the CB chemosensory responses to hypercapnia in a sex-dependent manner.

Soliz et al. (2007) found that transgenic mouse line (Tg6), with high plasma levels of human Epo, did not show significant differences in resting minute ventilation compared to wild-type mice. Present results showed that Epo injections did not change CB chemosensory discharges in normoxia. Then, our results suggest that Epo is not related to the regulation of the tonic CB activity in normoxia. Therefore, agrees and extend previous studies showing no effect of Epo on breathing regulation under normoxic conditions.

It has been shown that Epo may regulate the ventilatory response to hypoxia in humans and mice (Jeton et al. 2017; Pichon et al. 2016; Soliz 2013; Soliz et al. 2012; Voituron et al. 2014). However, we did not observe significant differences before and after Epo injection on the CB chemosensory response to acute hypoxia. Furthermore, no sex differences were found on the CB chemosensory response to hypoxia. It is

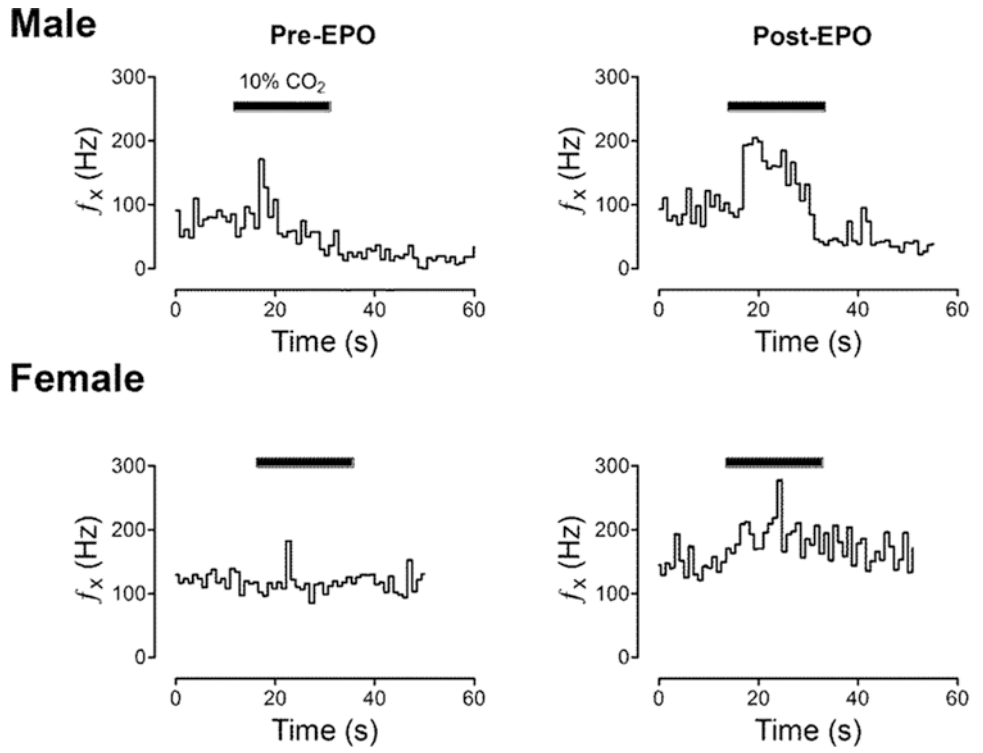

Fig. 12.3 Epo modulate the CB chemosensory responses to hypercapnia. Representative carotid sinus nerve recordings obtained in one male rat during acute (20s) hypercapnic stimulation (10%), before and after Epo i.v. injection (Upper panel) and one representative recording obtained in one female rat (Lower panel) during hypercapnia, before and after Epo administration

important to note that we only measure the effects of Epo on the CB chemoreceptor activity in response to hypoxia but not the chemoreflex-mediated hypoxic ventilatory response. Indeed, it has been proposed that Epo may regulate central brainstem areas related to chemoreflex control of the ventilation (i.e. NTS). Then, it is plausible that Epo may regulate the HVR through a central mechanism rather than a peripheral modulation of CB chemoreceptors.

In addition, we found that Epo modulate the CB response to hypercapnia in a sex-dependent manner. Indeed, in female rats Epo injections results in an increase CB chemosensory response to CO_2 without changing the duration of the response. In contrast, in male rats Epo only elicit increase in the duration of the CB chemosensory response to hypercapnia. Together, our data suggest that Epo modulate the CB nerve activity in response to hypercapnia acting on different EpoR expressed in male CBs compared to female CBs. Future studies are needed to address the differences, if any, on the expression profiles of EpoR on male and female CBs.

In summary our data suggest that Epo could modulate the CB chemosensory response to hypercapnia, but not to hypoxia in a sex-dependent manner. Additionally, Epo injections did not affects baseline CB chemosensory discharge in normoxia nor in male nor in female rats, suggesting that Epo did not contribute to tonic CB activity.

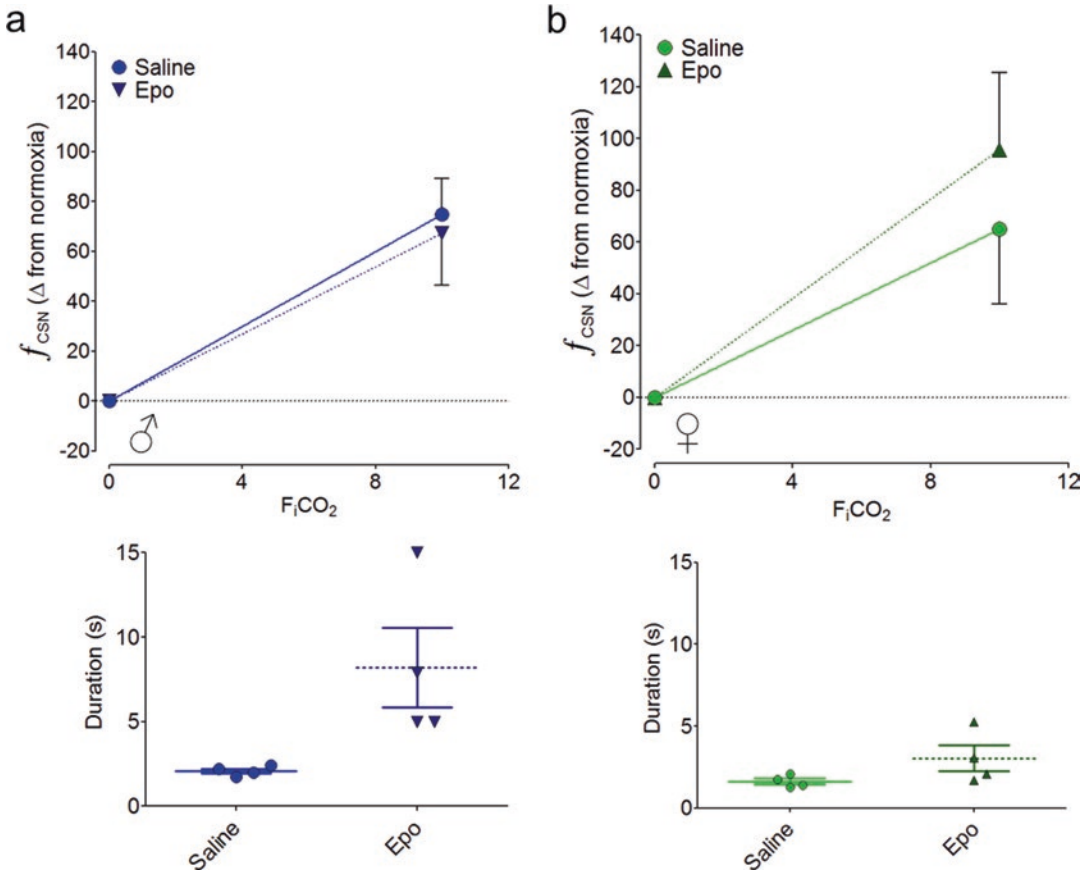

Fig. 12.4 Epo effects on CB chemosensory response to hypercapnia in adult male and female rats. *In situ* carotid sinus nerve recordings were obtained in spontaneously breathing animals during acute (20s) hypercapnic simulation. No effect of Epo was found on CB maximal response to hypercapnia in male rats (**a**, Upper panel). However, Epo administration results in a large increase in the duration of the response to hypercapnia (**a**, Lower panel). In contrast, Epo increases the CB response to hypercapnia (10%) in female rats (**b**) without affecting the duration of the CB response to hypercapnia (**b**, Lower panel). *, $P < 0.05$, Student t-test, n = 4 per group

Acknowledgements This work was supported by an ECOS-SUD program (N.V. action n°C16S03 and Programa de cooperación ECOS-CONICYT C16S03 to R.D.R.) and by Fondecyt grants #1140275 and #1180172 to R.D.R. and #1150040 to R.I. The "Relations Internationales" and the "Invited Professor" programs of the University Paris 13 also supported this study.

References

Berkenbosch A, Olievier CN, DeGoede J, Kruyt EW (1991) Effect on ventilation of papaverine administered to the brain stem of the anaesthetized cat. J Physiol 443:457–468

Berkenbosch A, Teppema LJ, Olievier CN, Dahan A (1997) Influences of morphine on the ventilatory response to isocapnic hypoxia. Anesthesiology 86:1342–1349

Del Rio R, Moya EA, Iturriaga R (2010) Carotid body and cardiorespiratory alterations in intermittent hypoxia: the oxidative link. Eur Respir J 36:143–150. https://doi.org/10.1183/09031936.00158109

Del Rio R, Munoz C, Arias P, Court FA, Moya EA, Iturriaga R (2011) Chronic intermittent hypoxia-induced vascular enlargement and VEGF upregulation in the rat carotid body is not prevented by antioxidant treatment. Am J Physiol Lung Cell Mol Physiol 301:L702–L711. https://doi.org/10.1152/ajplung.00128.2011

Despas F, Xhaët O, Senard JM, Verwaerde P, Jourdan G, Curnier D, Galinier M, Pathak A (2006) Chemoreflexes from physiology to practice. MT Cardio 2:321–327

Digicaylioglu M, Bichet S, Marti HH, Wenger RH, Rivas LA, Bauer C, Gassmann M (1995) Localization of specific erythropoietin binding sites in defined areas of the mouse brain. Proc Natl Acad Sci U S A 92:3717–3720

Feldman JL, Mitchell GS, Nattie EE (2003) Breathing: rhythmicity, plasticity, chemosensitivity. Annu Rev Neurosci 26:239–266. https://doi.org/10.1146/annurev.neuro.26.041002.131103

Gonzalez C, Almaraz L, Obeso A, Rigual R (1992) Oxygen and acid chemoreception in the carotid body chemoreceptors. Trends Neurosci 15:146–153

Gonzalez C, Vicario I, Almaraz L, Rigual R (1995) Oxygen sensing in the carotid body. Biol Signals 4:245–256

Guyenet PG (2014) Regulation of breathing and autonomic outflows by chemoreceptors. Comp Physiol 4:1511–1562. https://doi.org/10.1002/cphy.c140004

Jelkmann W (1992) Erythropoietin: structure, control of production, and function. Physiol Rev 72:449–489

Jelkmann W (2011) Regulation of erythropoietin production. J Physiol 589:1251–1258. https://doi.org/10.1113/jphysiol.2010.195057

Jeton F, Soliz J, Marchant D, Joseph V, Richalet JP, Pichon A, Voituron N (2017) Increased ventilation in female erythropoietin-deficient mouse line is not progesterone and estrous stage-dependent. Respir Physiol Neurobiol 245:98–104. https://doi.org/10.1016/j.resp.2017.07.002

Lam SY, Tipoe GL, Fung ML (2009) Upregulation of erythropoietin and its receptor expression in the rat carotid body during chronic and intermittent hypoxia. Adv Exp Med Biol 648:207–214. https://doi.org/10.1007/978-90-481-2259-2_24

Lopez-Barneo J, Gonzalez-Rodriguez P, Gao L, Fernandez-Aguera MC, Pardal R, Ortega-Saenz P (2016) Oxygen sensing by the carotid body: mechanisms and role in adaptation to hypoxia. Am J Physiol Cell Physiol 310:C629–C642. https://doi.org/10.1152/ajpcell.00265.2015

Pichon A et al (2016) Erythropoietin and the use of a transgenic model of erythropoietin-deficient mice. Hypoxia 4:29–39. https://doi.org/10.2147/HP.S83540

Prabhakar NR, Peers C (2014) Gasotransmitter regulation of ion channels: a key step in O2 sensing by the carotid body. Physiology 29:49–57. https://doi.org/10.1152/physiol.00034.2013

Smith CA, Blain GM, Henderson KS, Dempsey JA (2015) Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to CO2: role of carotid body CO2. J Physiol 593:4225–4243. https://doi.org/10.1113/JP270114

Soliz J (2013) Erythropoietin and respiratory control at adulthood and during early postnatal life. Respir Physiol Neurobiol 185:87–93. https://doi.org/10.1016/j.resp.2012.07.018

Soliz J et al (2005) Erythropoietin regulates hypoxic ventilation in mice by interacting with brainstem and carotid bodies. J Physiol 568:559–571. https://doi.org/10.1113/jphysiol.2005.093328

Soliz J, Soulage C, Hermann DM, Gassmann M (2007) Acute and chronic exposure to hypoxia alters ventilatory pattern but not minute ventilation of mice overexpressing erythropoietin. Am J Physiol Regul Integr Comp Physiol 293:R1702–R1710. https://doi.org/10.1152/ajpregu.00350.2007

Soliz J, Khemiri H, Caravagna C, Seaborn T (2012) Erythropoietin and the sex-dimorphic chemoreflex pathway. Adv Exp Med Biol 758:55–62. https://doi.org/10.1007/978-94-007-4584-1_8

Voituron N et al (2014) Catalyzing role of erythropoietin on the nitric oxide central pathway during the ventilatory responses to hypoxia. Physiol Rep 2:e00223. https://doi.org/10.1002/phy2.223