Respiratory Physiology & Neurobiology 242 (2017) 73–79

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Pharmacological, but not genetic, alteration of neural Epo modifies the CO_2/H^+ central chemosensitivity in postnatal mice

Sofien Laouafa[a,e], Anne-Sophie Perrin-Terrin[c,d], Florine Jeton[c], Elizabeth Elliot-Portal[a,b], Rose Tam[a], Laurence Bodineau[d], Nicolas Voituron[c,1], Jorge Soliz[a,b,*,1]

[a] Université Laval, Faculté de Médecine, Centre de Recherche Institut universitaire de cardiologie et de pneumologie de Québec, Département de Pédiatrie, Québec, QC, Canada
[b] Molecular biology and Biotechnology Institute, Universidad Mayor de San Andres, La Paz, Bolivia
[c] Université Paris 13, Sorbonne Paris Cité, UFR SMBH, Laboratoire "Hypoxie et poumons", EA 2363, 93017 Bobigny, France
[d] Sorbonne Universités, UPMC Univ Paris 06, INSERM, UMR_S1158 Neurophysiologie Respiratoire Expérimentale et Clinique, F-75013, Paris, France
[e] LEHNA, UMR CNRS 5023, Ecologie des Hydrosystèmes Naturels et Anthropisés, Université de Lyon, Université Lyon 1, ENTPE, 6 rue Raphael Dubois, 69622 Villeurbanne, France

ARTICLE INFO

Keywords:
Newborn mice
Erythropoietin
Soluble erythropoietin receptor
Respiration
Central chemosensitivity
Hypercapnia
ERK/Akt

ABSTRACT

Cerebral erythropoietin (Epo) plays a crucial role for respiratory control in newborn rodents. We showed previously that soluble Epo receptor (sEpoR: an Epo antagonist) reduces basal ventilation and hypoxic hyperventilation at postnatal day 10 (P10) and in adult mice. However, at these ages (P10 and adulthood), Epo had no effect on central chemosensitivity. Nevertheless, it is known that the sensitivity to CO_2/H^+ during the mammalian respiratory network maturation process is age-dependent. Accordingly, in this study we wanted to test the hypothesis that cerebral Epo is involved in the breathing stimulation induced by the activation of central CO_2/H^+ chemoreceptors at earlier postnatal ages. To this end, *en bloc* brainstem-spinal cord preparations were obtained from P4 mice and the fictive breathing response to CO_2-induced acidosis or metabolic acidosis was analyzed. This age (P4) was chosen because previous research from our laboratory showed that Epo altered (in a dose- and time-dependent manner) the fictive ventilation elicited in brainstem-spinal cord preparations. Moreover, as it was observed that peripheral chemoreceptors determined the respiratory sensitivity of central chemoreceptors to CO_2, the use of this technique restricts our observations to central modulation. Our results did not show differences between preparations from control and transgenic animals (Tg21: overexpressing cerebral Epo; Epo-TAg^h: cerebral Epo deficient mice). However, when Tg21 brainstem preparations were incubated for 1 h with sEpoR, or with inhibitors of ERK/Akt (thus blocking the activation of the Epo molecular pathway), the fictive breathing response to CO_2-induced acidosis was blunted. Our data suggest that variation of the Epo/sEpoR ratio is central to breathing modulation during CO_2 challenges, and calls attention to clinical perspectives based on the use of Epo drugs at birth in hypoventilation cases.

1. Introduction

It has become clear over the last decade that erythropoietin (Epo) is extensively produced in the brain (by neurons and astrocytes) (Rabie and Marti, 2008). Moreover, the expression of Epo and its receptor (EpoR) in rodent (Knabe et al., 2004) and human (Juul et al., 1998) brains starts during early gestation (1 week and 5 weeks, respectively) and is maintained (albeit with modulated variations) throughout life. Accordingly, we now know that Epo plays a critical role in the development, maintenance, protection and repair of the nervous system (Chen et al., 2007; Kumral et al., 2011; Silva et al., 1996; Sola et al., 2005; Yu et al., 2002). Research from our laboratories also contributed to this new concept of Epo. By using transgenic mice showing over- or deficient-expression of cerebral Epo, we reported altered modulation of basal ventilation and hypoxic hyperventilation, both at postnatal and adult ages (Ballot et al., 2015a; Caravagna et al., 2015; El Hasnaoui-Saadani et al., 2009; Khemiri et al., 2011; Macarlupu et al., 2006; Pichon et al., 2016b; Soliz et al., 2005; Voituron et al., 2014). Moreover, the basal ventilation and hypoxic ventilatory response were also drastically reduced (by about 50%) when the Epo/sEpoR ratio was

* Corresponding author at: Faculté de Médecine, Université Laval, Institut universitaire de cardiologie et de pneumologie de Québec, 2725 chemin Ste-Foy, Québec (Québec), G1V 4G5 Canada.
E-mail address: jorge.soliz@criucpq.ulaval.ca (J. Soliz).
[1] Both senior authors contributed equally to this work.

http://dx.doi.org/10.1016/j.resp.2017.04.003
Received 9 December 2016; Received in revised form 24 March 2017; Accepted 5 April 2017
Available online 08 April 2017

altered by injecting soluble EpoR (sEpoR, the natural competitive antagonist of Epo) in the cisterna magna (intra-cisternal injection) of WT mice (Ballot et al., 2015b). In line with these findings, ubiquitous expression of Epo and EpoR were found in brainstem areas controlling ventilation, including the preBötzinger complex, the nucleus tractus solitarii, and catecholaminergic groups of the brainstem (Soliz et al., 2005).

Taking into account all these results, and keeping in mind that the brainstem is the main structure containing the CO_2/H^+ chemosensors (Guyenet et al., 2010; Guyenet et al., 2008; Nattie, 1999), it was surprising to find that cerebral Epo does not affect central CO_2/H^+ chemosensitivity in mice. Previous studies from our laboratory showed that the hypercapnic ventilatory response was unaffected in postnatal day 10 (P10) and adult mice receiving an intra-cisternal injection of sEpoR (Ballot et al., 2015b). Similarly, the hypercapnic ventilatory response was found to be unaffected in adult transgenic mice over-expressing Epo only in the brain (Tg21) (Laouafa et al., 2016a). It is important to recall, however, that the sensitivity to CO_2/H^+ during maturation of the mammalian respiratory network is age-dependent (Abu-Shaweesh et al., 1985; Bissonnette and Knopp, 2004; Frantz et al., 1976; Putnam et al., 2005; Rigatto et al., 1975). In fact, the stimulation of central CO_2/H^+ chemoreceptors is considered essential for breathing at neonatal ages (Ramanantsoa et al., 2011), and there are diseases such as congenital central hypoventilation syndrome (Weese-Mayer et al., 2010) characterized by a lack of ventilatory response to inhaled CO_2 and elevated PCO_2. Accordingly, in this study we wanted to investigate in mice whether cerebral Epo modulates the central ventilatory response to CO_2/H^+ at ages earlier than P10. To this aim, we used P4 mice showing over- and deficient-expression of cerebral Epo. This age was chosen because previous research from our laboratory showed that at P4 Epo alters (in a dose- and time-dependent manner) the fictive breathing in mice (Khemiri et al., 2011). Moreover, as it was reported that peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to CO_2 (hyperadditive effect; (Smith et al., 2015)), experiments were performed on brainstem-spinal cord preparations to restrict observations to the medullary CO_2/H^+ chemoreceptors. After dissection, preparations were exposed to CO_2-induced acidosis or metabolic acidosis as previously described by our lab (Joubert et al., 2016; Voituron et al., 2010a; Voituron et al., 2010b). Experiments challenging the pharmacological decrease of the Epo availability or the restriction of Epo molecular signaling were also performed on these preparations. Interestingly, our results showed that pharmacological, but not genetic, modulation of Epo altered the postnatal central CO_2/pH chemosensitivity at postnatal day P4, a period during which the respiratory control centers of mice are actively completing development and maturation (Viemari et al., 2003).

2. Material and methods

2.1. Animals

The transgenic Tg21 mouse strain that overexpresses Epo in the brain was obtained by a generous donation from Professor Max Gassmann, University of Zurich, Switzerland. A detailed description of the Tg21 strain was reported previously (Ruschitzka et al., 2000; Wiessner et al., 2001). In brief, Tg21 mice were created from the C57BL/6 mouse line. In our laboratory, Tg21 heterozygous mice were backcrossed with C57Bl/6 mice for more than six generations to obtain the corresponding control mice used in this study. For the experimentation only male Tg21 homozygotes were used.

Epo deficient (Epo-TAg^h) mice present a targeted disruption in the 5′ untranslated region of the Epo gene that reduces whole body Epo expression (Binley et al., 2002). A detailed description of the Epo-TAg^h strain was reported previously (Pichon et al., 2016a). In brief, the Epo-TAg^h mice showed an Epo concentration of about 50 pg/ml (WT 150 pg/ml) in plasma, and 0.10 pg/mg (WT 0.40 pg/mg) in the brain (El Hasnaoui-Saadani et al., 2009).

All mice were housed and bred in a room maintained at constant temperature and humidity on a 12-h light cycle (07:30–19:30). Food and tap water were provided ad libitum. Animal experiments with Tg21 mice were approved by the Laval University Animal Ethics Committee (Protocol #10-085-2) and carried out in accordance with the EU Directive 2010/63/EU and the Uniform Requirements for Manuscripts submitted to Biomedical journals (URM). Experimental protocols for Epo-TAg^h mice were approved by the Ethics Committee for Animal Experiment Charles Darwin (Ce5/2011/05), done in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EU) for animal care, and conducted in accordance with the French legislation for animal care.

2.2. In vitro brainstem–spinal cord preparations

Experiments were performed on isolated *en bloc* brainstem–spinal cord preparations obtained from mice at P4 (n = 10–11/group). Newborns were cryoanesthetized by total immersion in ice for 4–5 min (Danneman et al., 1997). As previously described (Smith et al., 1990; Viemari et al., 2003), the brainstem–spinal cord was isolated from the brain, and the pons was subsequently removed from the preparation (Khemiri et al., 2011; Viemari and Hilaire, 2002). Inhibition from the pons region over medullary network activity in mice is strong, making removal of the pons necessary to allow spontaneous activity in the respiratory network (Smith et al., 1990; Viemari et al., 2003) 2003). The preparation was superfused in a recording chamber with artificial cerebrospinal fluid (aCSF: 129 mM NaCl, 3.35 mM KCl, 1.26 mM $CaCl_2$, 1.15 mM $MgCl_2$, 21.0 mM $NaHCO_3$, 0.58 mM NaH_2PO_4, and 30.0 mM glucose; final pH value in gassed aCSF, 7.3–7.4) bubbled with carbogen (95% O_2, 5% CO_2). Temperature was maintained at 26°C (Temperature Controller TC-324B, Warner Instruments, Hamden, CT).

2.3. Electrophysiological recordings

Electrophysiological recordings were obtained according to a previously described protocol (Belzile et al., 2007; Khemiri et al., 2011; Kinkead et al., 2002). Respiratory-related activity of phrenic motoneuron axons of the C4 ventral root were recorded using a suction electrode (model 573000; A-M Systems, Everett, WA) after a superfusion equilibration period of 20 min, allowing the signal to reach a steady-state. The signal was amplified (gain = 10,000) and filtered (low cut-off, 10 Hz; high cut-off, 5 kHz) using a differential AC amplifier (model 1700; A-M Systems, Everett, WA, in Canada; GRASS P 511G, in France). Raw signals were integrated using a Paynter filter (Moving Averager model MA- 821; CWE, Ardmore, PA in Canada; or a custom-made Channel Integrator, in France), and recorded in a digitized form with a data acquisition system (model DI-720; Dataq Instruments, Akron, OH or CED, Cambridge, UK). The sampling rate for the analog-digital conversion of the raw signal was 2.5 kHz.

2.4. CO_2-induced acidosis versus metabolic acidosis

Two different forms of acidosis were used in this study: CO_2-induced and metabolic (see detailed protocol below). The reason for this was that similar studies investigating the impact of cerebral Epo in the neural control of ventilation at P4 were being performed independently (and at the same time) in two different lab teams. Specifically, CO_2-induced acidosis was used to perform experiments in Tg21 mice (overexpressing Epo in the brain) in Canada, while metabolic acidosis was used to perform experiments in EpoTAg^h mice (showing deficient expression of Epo in the brain), in France. As the results obtained individually in both labs were complementary and arrive at the same conclusion, we decided to report them together in one manuscript.