Respiratory Physiology & Neurobiology 267 (2019) 12–19

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Developmental expression patterns of erythropoietin and its receptor in mouse brainstem respiratory regions

Edith M. Schneider Gasser[b], Elizabeth Elliot-Portal[a], Christian Arias-Reyes[a,d], Karen Losantos-Ramos[a], Kasifa Khalid[b], Omolara Ogunshola[c], Jorge Soliz[a,d,*]

[a] Institut universitaire de cardiologie et de pneumologie de Québec, Centre Hospitalier Universitaire de Québec (CHUQ), Faculty of Medicine, Université Laval, Québec, QC, Canada
[b] Institute of Pharmacology and Toxicology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
[c] Institute of Veterinary Physiology and Zurich Center of Integrative Human Physiology (ZIHP), Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland
[d] Instituto de Biología Molecular y Biotecnología, Facultad de Ciencias Puras y Naturales, Universidad Mayor de San Andrés, La Paz, Bolivia

ARTICLE INFO

Keywords:
Cytokines
Pre-bötzinger complex
Catecholaminergic nuclei
Brain development

ABSTRACT

Erythropoietin (EPO) is a hypoxia-inducible hormone, classically known to enhance red blood cell production upon binding its receptor (EPOR) present on the surface of the erythroid progenitor cells. EPO and its receptor are also expressed in the central nervous system (CNS), exerting several non-hematopoietic actions. EPO also plays an important role in the control of breathing. In this review, we summarize the known physiological actions of EPO in the neural control of ventilation during postnatal development and at adulthood in rodents under normoxic and hypoxic conditions. Furthermore, we present the developmental expression patterns of EPO and EPORs in the brainstem, and with the use of in situ hybridization (ISH) and immunofluorescence techniques we provide original data showing that EPOR is abundantly present in specific brainstem nuclei associated with central chemosensitivity and control of ventilation in the ventrolateral medulla, mainly on somatostatin negative cells. Thus, we conclude that EPO signaling may act through glutamatergic neuron populations that are the primary source of rhythmic inspiratory excitatory drive. This work underlies the importance of EPO signaling in the central control of ventilation across development and adulthood and provides new insights on the expression of EPOR at the cellular level.

1. Introduction

Erythropoietin (EPO) was discovered as a kidney-derived hormone essential for the survival and differentiation of erythroid precursor cells (Bauer, 1995; Jelkmann, 1994b). Since Sasaki and his co-workers reported the presence of a functional EPO receptor (EPOR) in PC12 cells (Masuda et al., 1993), EPO was shown to be expressed in several other tissues, including the brain (Bernaudin et al., 1999; Digicaylioglu et al., 1995; Gassmann et al., 2003; Marti, 2003). EPO and EPORs have been detected in several brain regions in rodents and primates, such as the cortex, hippocampus, amygdala, cerebellum, hypothalamus, and caudate nucleus (Siren and Ehrenreich, 2001). Subsequently, it was recognized that EPO in these regions has anti-apoptotic, anti-cytotoxic, anti-oxidative, and anti-inflammatory functions (Byts and Siren, 2009). In addition to its neuroprotective actions, EPO has been shown to promote maintenance of the blood-brain barrier, and also angiogenesis, neurogenesis, and the migration of immature neurons (Byts and Siren, 2009; Kumral et al., 2010). Furthermore, it was shown that EPO prevents mitochondrial dysfunction after traumatic brain injury, thereby maintaining the mitochondrial membrane potential and calcium homeostasis (Millet et al., 2015). In line with this observation, experiments in rodents showed that EPO significantly reduces spatial learning deficits by increasing antioxidant defenses in the hippocampus (Al-Qahtani et al., 2014) and reverses cognitive and behavioral morbidities by reducing NADPH oxidase activity (Dayyat et al., 2012). We (Seaborn et al., 2011; Soliz et al., 2005) and others (Oshima et al., 2018) have shown a physiological role for EPO in the control of breathing, by demonstrating that EPO stimulates ventilation in normoxic and hypoxic conditions interacting with respiratory and catecholaminergic centers in the brainstem and the carotid bodies. The period of respiratory development has been proposed as of major risk for sudden infant death syndrome (Filiano and Kinney, 1994) and apneas, thus our work has

* Corresponding author at: Institut Universitaire de Cardiologie et de Pneumologie de Québec (IUCPQ), 2725, chemin Sainte-Foy, Québec, QC, G1V 4G5, Canada.
E-mail address: jorge.soliz@crchuq.ulaval.ca (J. Soliz).

https://doi.org/10.1016/j.resp.2019.05.012
Received 28 February 2019; Received in revised form 23 April 2019; Accepted 20 May 2019
Available online 30 May 2019
1569-9048/

also expanded to evaluate the control of ventilation by EPO during the postnatal developmental window, and showed that EPO does improve the ventilatory response to hypoxia, adding a potential role for EPO in perinatal neuroprotection (Caravagna et al., 2014, 2015; Caravagna and Soliz, 2015). In general, however, the expression of EPO and EPOR in the regions of respiratory control remain poorly understood. In this review, we focus on the physiological effects of EPO in the control of ventilation across development and at adulthood under normoxic and hypoxic conditions, and the expression of EPO and EPOR mRNA. We provide new findings on EPOR localization in the brainstem ventrolateral medulla, the region where the neural control of respiratory activities occurs.

2. EPO's impact in the neural control of ventilation at adult ages

Apart from pathological conditions, studies performed in our and other laboratories demonstrated that EPO in the brain also exerts physiological functions. We have clearly shown that EPO prevents hypoxia-induced respiratory depression at adulthood (Ballot et al., 2015; Soliz et al., 2007a; Soliz and Joseph, 2005; Soliz et al., 2007b). By using transgenic mice overexpressing EPO in the brain only (termed Tg21) we showed that EPO facilitates the ventilatory response to hypoxia. In line with this finding, the ventilatory response to acute hypoxia of chemodenervated animals revealed that WT, but not Tg21 mice exhibited life-threatening apneas. These data indicate that cerebral EPO maintains high hypoxic respiratory activity despite the lack of sensory information from peripheral chemoreceptors (Soliz et al., 2006, 2005). Furthermore, we observed that ventilatory acclimatization to sustained hypoxia (3 days at 10% O_2) is also facilitated by overexpression of cerebral EPO, and when cerebral EPO is antagonized (by intracisternal injection of the soluble EPO receptor – sEPOR), normoxic ventilation and hypoxic ventilatory response are decreased (Ballot et al., 2015), and the ventilatory acclimatization is abolished. In agreement with these findings, further experiments revealed that EPO-mediated modulation of sensory integration involved, at least in part, the modulation of catecholamine synthesis in the brainstem. In fact, immunostaining studies revealed the presence of EPOR in the pons (A5, and A6) and medulla (A1C1, and A2C2); catecholaminergic analyses showed altered activity of tyrosine hydroxylase (Soliz et al., 2005). Finally, we recently demonstrated in mice that cerebral EPO protects against the deleterious consequences of sleep-disorder breathing (SDB). SDB is characterized by repeated episodes of cessation of breathing followed by repeated arterial oxygen desaturation and bradycardia, leading to chronic intermittent hypoxia. In turn, IH leads to general oxidative imbalance, resulting in a short and long-lasting alteration of chemical structures of proteins, fatty acids, and DNA. In comparison with other tissues, the brain is especially vulnerable to oxidative stress because it consumes a large fraction of total oxygen (20%), has low antioxidant enzyme activity, possess high concentrations of metal ions capable of forming hydroxyl radicals (Fenton reaction), and is rich in polyunsaturated fatty acids (Lagranha et al., 2017; Popa-Wagner et al., 2013). By using Tg21 animals, we showed that cerebral EPO prevents oxidative damage in the brainstem, thus avoiding cardiorespiratory disorders induced by CIH (Elliot-Portal et al., 2018).

3. EPO's impact in the neural control of ventilation at neonatal ages

The neonatal period is especially critical in mammals due to the fact that respiration is not fully mature at birth (Bissonnette, 2000). Indeed, the development of the neural control of ventilation begins early during gestation (Carroll and Agarwal, 2010; Viemari et al., 2003) but, although being functional at birth, it is still unstable in newborns with frequent respiratory pauses, and requires weeks or months in humans to complete the maturation process (Carroll and Agarwal, 2010). As such, interruption of breathing at neonatal ages prevents gas exchange and results in systemic hypoxia that, if prolonged, can lead to cardio-respiratory failure or sudden infant death. Experiments performed in our laboratory showed strong evidence that EPO at neonatal ages accelerates the maturation of the neural networks controlling ventilation. Using brainstem-spinal cord preparations, we showed that EPO attenuated the hypoxia-induced respiratory depression, characterized by a decline in the respiratory bursting activity. Furthermore, the maturation of the respiratory command in brainstem-spinal cord preparations from Tg21 mice showed a decreased burst frequency and an increased duration at P0 similar to the motor pattern of control mice at age P3-P4 (Caravagna et al., 2014). Maturation of respiratory control is characterized by a lower burst frequency and longer bursting with age (Viemari et al., 2003), therefore EPO accelerated the maturation of the respiratory control. Additionally, in vivo experiments in Tg21 mice, showed an increased ventilatory response to hypoxia at postnatal days P3, P7, and P21 (Caravagna et al., 2015). Altogether, these findings strongly suggest that EPO promotes a more rapid maturation of the brain at neonatal ages by increasing the neural ability to tolerate physiological and pathological levels of oxygen deprivation. In line with our data, it was recently reported in neonatal (0- to 5-old) Wistar rats that EPO is produced in response to hypoxia in rostroventrolateral medulla (RVLM) neurons, promoting the increase of blood pressure (Oshima et al., 2018). Furthermore, histological examination showed that EPORs are present in bulbospinal RVLM neurons and some co-expressed with TH immunoreactivity, suggesting that catecholaminergic RVLM neurons at neonatal ages express EPORs (Oshima et al., 2018). We performed further experiments to determine whether EPO can be used as a novel therapeutic tool against apnea of prematurity (AoP). Owing to the immaturity of the brain, AoP is recurrent in infants born with less than 34 weeks of gestational age. AoP is associated with severe and repeated episodes of neonatal intermittent hypoxia (nIH) leading, in turn, to increased respiratory instability, augmentation of apneic episodes, increased morbidity, prolonged hospitalization, and long-term cognitive and neurodevelopmental outcomes (de Lima et al., 2005; Jobe and Kallapur, 2010). In fact, studies at the cellular level showed that nIH increases the production of reactive oxygen species (ROS), and decreases anti-oxidative defenses (Di Fiore et al., 2013, 2016). Caffeine is an adenosine receptor antagonist that increases central respiratory drive, thus reducing the frequency of apneas. As such, caffeine is the most widely used treatment against AoP (Di Fiore et al., 2016). However, caffeine is not completely efficient, as about half of treated AoP infants still present an elevated frequency of apneic events (Erenberg et al., 2000). By using rat pups exposed to nIH (from postnatal ages 3 to 10) we showed that EPO and caffeine have a similar protective effect in preventing the increase of apneas. Interestingly, our data showed that caffeine achieved this task by preventing the increase of pro-oxidative enzymes (NADP oxidase - NOX). EPO's mechanism involved the upregulation of antioxidative molecules (superoxide dismutase - SOD). In fact, these findings crucially suggest that EPO can be used as a new therapeutical tool against AoP (Laouafa et al., 2019).

4. Developmental expression pattern of EPO in the neonatal brainstem

EPO and its receptor are extensively expressed in the fetal human (Juul et al., 1998b) and rodent (Kumral et al., 2011) brain. Such expression, in humans, starts at 5–10 weeks post-conception at the periventricular (germinal), ventricular and subventricular zones, and becomes prominent in neurons and astrocytes about 20–32 weeks post-conception (Juul et al., 1998b), then decreases during neonatal ages (Ehrenreich et al., 2005; Knabe et al., 2004). So far, however, the developmental expression pattern of EPO in brainstem respiratory areas has not yet been described. By using the radioimmunoassay (RIA) technique, we determined the age-dependent expression of EPO in the postnatal brainstem (medulla oblongata and pons) and the remaining portion of the brain (midbrain and forebrain) (Fig. 1). EPO protein

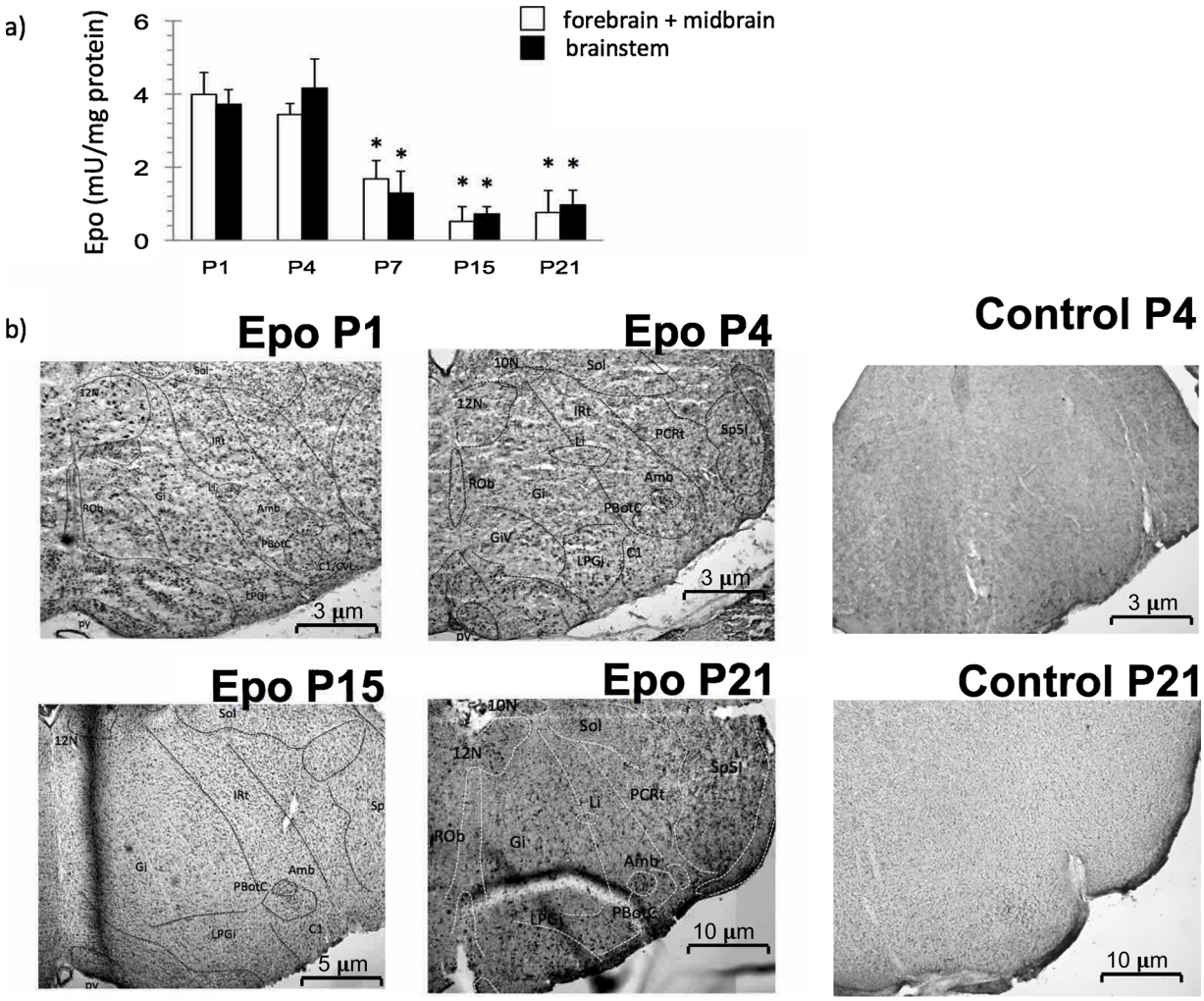

Fig. 1. Age-dependent expression of EPO protein in the mouse brain during the postnatal development at P1, P4, P7, P15, and P21 as **a)** measured in the brain by radioimmunoassay and **b)** measured in the brainstem Pre-Bötzinger complex (PBotC) region by immunohistochemistry (EPO = black dots). Ambiguus (Amb); Dorsal motor of the vagus (10 N); Gigantocellular reticular (Gi); Hypoglossal (12 N); Inferior olive (IO); Lateral paragigantocellular (LPGi); Lateral reticular nucleus (LRt); Parvicellular reticular (PCRt); Pyramidal tract (py); Raphe obscurus (ROb); Linear nucleous of the medulla (li); Solitary tract (sol); Spinal trigeminal, interpolar part (Sp51); C1 noradrenaline cells (C1); caudoventrolateral reticulum (CVL). References according to *Atlas of the developing mouse brain* (Paxinos et al., 2007) and *The mouse brain* atlas (Franklin & Paxinos, 2007). *$p < 0.05$ compared to EPO levels at P1.

concentration was determined by using ^{125}I-Epo-based radioimmunoassay (RIA; DiaSorin, Stillwater, MN, USA), according to previously published protocols (Wenger et al., 1998). Our results revealed that EPO levels in the brainstem are higher at early postnatal ages P1 and P4 (around 4mU/mg protein), reduces significantly to half at P7 (around 2 mU/mg protein) and continues to reduce by half at P14, remaining at that level until P21 (Fig. 1a). The expression level of EPO in the brainstem (medulla oblongata + pons) is comparable to the forebrain and midbrain (Fig. 1a). We determined by immunohistochemistry the localization of EPO in specific brainstem areas at postnatal ages P1, P4, P15, and P21. Brainstems were serially sliced at 20 µm and incubated overnight with a primary antibody against EPO (Cat# H-162 sc-7956, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1:200), and a secondary biotinylated rabbit anti-goat antibody (Cat# 305-065-045, Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA; 1:500). Our results revealed that EPO is ubiquitously expressed in the brainstem, including also the areas associated with respiratory control, such as the nucleus tractus solitarii (sol), the Pre-Bötzinger complex (pBötC), and the nucleus ambiguus (Amb) (Fig. 1b). EPO is also expressed in motor nuclei involved in the regulation of ventilatory output, such as the hypoglossal nucleus (12 N) and vagus (10 N) (Fig. 1b). EPO staining showed a higher number of positive cells in the brainstem at P1, in line with the RIA measurements. Control staining for EPO detection performed in the absence of primary EPO antibody was included for ages P4 and P21 (Fig. 1b).

5. Developmental expression pattern of EPOR in the neonatal brainstem

EPOR is a type I cytokine receptor that forms a homodimer following binding of one single EPO molecule (Jelkmann, 1994a). The binding of EPO to EPOR (which exists as a preformed dimer), induces a conformational change that brings constitutively associated Janus family tyrosine-protein kinase 2 (JAK2) molecules in close proximity and stimulates their activation by transphosphorylation. In turn, the EPOR activation promotes the phosphorylation of cytosolic domains, triggering several intracellular pathways (Jelkmann, 1994a). The expression of EPOR was evidenced in the embryonic mammalian brain (Knabe et al., 2004; Liu et al., 1994) and in neurons, astrocytes, oligodendrocytes, microglia and endothelial cells in the adult brain (Rabie & Marti, 2008). In the fetal human brain, EPOR's are already present at the fifth week of gestation and remain expressed (albeit undergoing modulations) throughout life (Juul et al., 1997, 1998b). Moreover, in fetal mouse, EPOR's are expressed in the yolk at E 9.5 (Suzuki et al.,

2002). In fact, EPO signaling is required for normal brain development, as its deficiency decreases neuronal progenitor cell number, increases apoptosis and leads to embryonic neurogenesis defects, among other effects (Alnaeeli et al., 2012). EPOR brain expression decreases by more than 1000-fold during development, reaching low levels at birth, which persists until adulthood (Juul et al., 1998a). Despite this abundant amount of work performed in the last 20 years, comparative studies questioned the specificity of commercially available EPOR antibodies (Elliott et al., 2006, 2010; Kirkeby et al., 2007). Thus, while the functional impact of EPO in the brain is not questioned, the localization of EPORs in the brain requires careful verification. We recently performed *in-situ* hybridization and immunofluorescence techniques to determine the developing expression pattern of EPOR's in brainstem RVLM. mRNA *in-situ* hybridization for murine EPOR was performed in parasagittal brainstem sections. Brainstem cryostat sections of 14 µm thickness and at 730 µm from the medial line were mounted on superfrost plus glass slides (Menzel GmbH & Co) and hybridization was done with DIG-labeled RNA hybridization probes at 37 °C. The RNA probe was a 500-bp fragment corresponding to exons 4 and 5 of the EPOR cDNA. Results of these experiments showed that EPOR mRNA is widely expressed all along the brainstem areas from P1 to P25 (Fig. 2a–d). Quantification of EPOR mRNA by quantitative PCR (Fig. 2e) showed that, contrary to EPO, the transcripts of EPOR in the brainstem increases at postnatal ages P14 and P25. However, while EPOR mRNA expression is similar to the rest of the brain at P1 and P7, its expression in comparison to major areas of the forebrain (olfactory bulb, hippocampus, cortex) becomes weaker at P14 and P25.

We used immunofluorescence techniques to determine the expression of EPORs in the ventrolateral medulla. Brainstem coronal slices taken between 360 µm to 600 µm from the brainstem were incubated overnight with a primary EPOR antibody (Cat# M-20 sc-697, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1:500), and Choline Acetyl Transferase (ChAT) (Cat# AB144 P, Millipore Corporation, Billerica, MA, USA; 1:500), or EPOR and Somatostatin (SST) (Cat# D-20 sc-7819, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1:250) (Joseph et al., 2006). Slices were then incubated 2 h at room temperature with secondary Fluorescein (FITC)-conjugated donkey anti-rabbit antibodies (Cat# 711-095-152, Jackson Immuno Research Laboratories Inc., West Grove, PA, USA; 1:500) for EPOR immunodetection, and secondary TexasRed-conjugated donkey anti-goat antibodies (Cat# GTX27123, Gene Tex, Irvine, CA, USA; 1:1000) for ChAT immunodetection and 1:100 for SST immunodetection. Our results showed that EPORs colocalize with most cells expressing ChAt (Fig. 3a) but only with some SST cells (Fig. 3b). In order to validate our results observed with immunofluorescence, we performed fluorescent ISH (FISH) in combination with immunofluorescence labeling of STT in parasagittal slices from wild type mice at P25 (Fig. 4). Our results showed that EPORs are abundantly expressed in the ventrolateral medulla in SST negative cells (Fig. 4c) but almost absent in SST positive cells (Fig. 4e). Thus, we conclude that EPO signaling may act through glutamatergic populations that are the primary source of rhythmic inspiratory excitatory drive.

6. Other putative EPOR in the brain

Apart from the classical EPOR, it was suggested that the action of EPO in neural tissue may be mediated by other types of receptors. Indeed, it has been revealed that exposure of primary cultures of neuronal cells from Coleoptera (beetles - *Tribolium castaneum*) and Orthoptera (grasshoppers - *Lacusta migratoria*) to 36 h of hypoxia ($\leq 1\%$ O_2) induced about 50% cell death, and that the treatment of such cells with human recombinant EPO (rhEpo) increased survival by about 20%, and led to neurons exhibiting longer neurites (Hahn et al., 2017; Ostrowski et al., 2011). As occurring in mammals, this effect was associated with an increased expression of antiapoptotic proteins induced by the activation of JAK2/STAT (but not PI3K) transduction pathways (Miljus et al., 2014, 2017). However, while a sequence comparison has not identified genes for EPO and EPOR in any insect species (Beschin

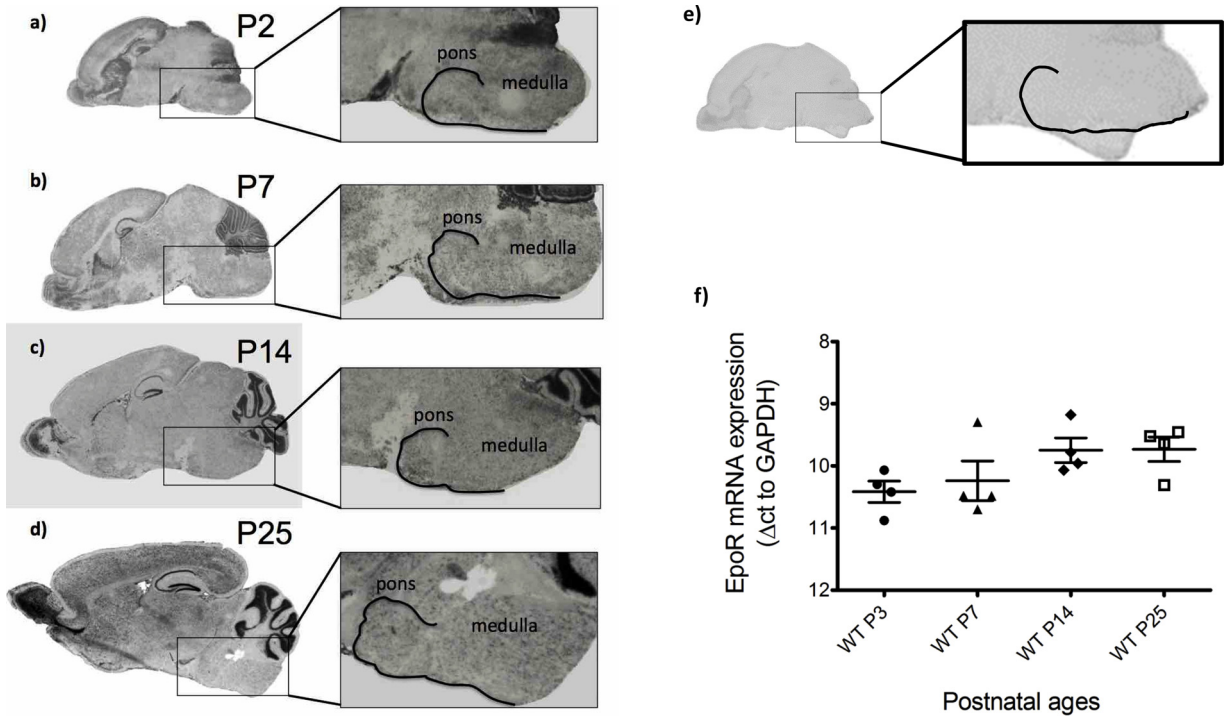

Fig. 2. Age-dependent expression of EPOR mRNA in the mouse brainstem evaluated by the *in-situ* hybridization (ISH) technique. Brain samples were obtained at ages of P2 (a), P7 (b), P14 (c), and P25 (d). Expression of EPOR mRNA is spread all along the brainstem sections, with stronger intensity with respect to the rest of the brain at ages P2 (a) and P7 (b). (e) ISH with a sense probe showing the background signal at P2. Quantitative PCR (qPCR) shows an increase in the EPOR transcript in the brainstem at P14 and P25 (f).

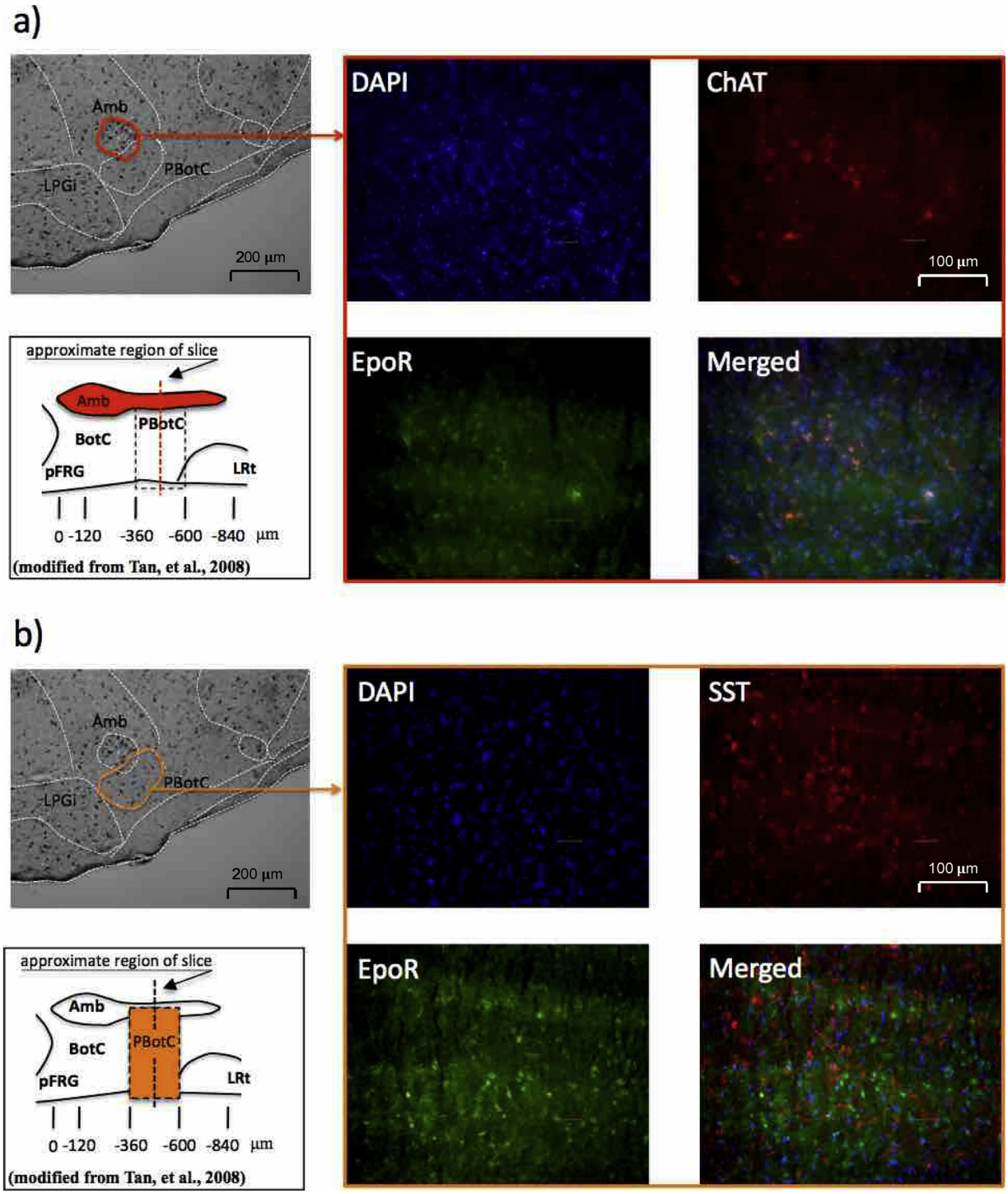

Fig. 3. EPOR expression in brainstem areas at postnatal day 25. **a)** EPOR's (green dots) mostly co-localize with choline acetyltransferase (ChAT; red dots), a marker of the nucleus ambiguus (Amb). **b)** EPOR's (green dots) do not co-localize with somatostatin (SST; red dots) in the PreBötzinger complex (PBotC). DAPI (blue dots). Lateral paragigantocellular (LPGi); Lateral reticular nucleus (LRt) Botzinger complex (Bot); Parafacial respiratory group (pFRG). References according to *The mouse brain* atlas (Franklin & Paxinos, 2007).

et al., 2001), it has been proposed that the spatial organization of a few critical amino acid residues, rather than the similarity of the complete sequence of a cytokine, is important for receptor binding (Heinrich et al., 2017). In line with this theory, it was shown that the insect ortholog (homologous genes that arose through speciation) of the human orphan cytokine receptor CRLF3 is a neuroprotective EPOR in *Tribolium castaneum* (Hahn et al., 2017). Remarkably, CRLF3 is a well-conserved gene throughout evolution, and orthologs of CRLF3 were reported in mammals, frog, fish, non-vertebrate chordates and some insects (Wyder et al., 2007). In fact, new experiments are now required to prove whether CRLF3 receptors are responsible for EPO neuroprotective actions in the brainstem. However, our data support the presence of the classical EPOR in the brainstem respiratory regions.

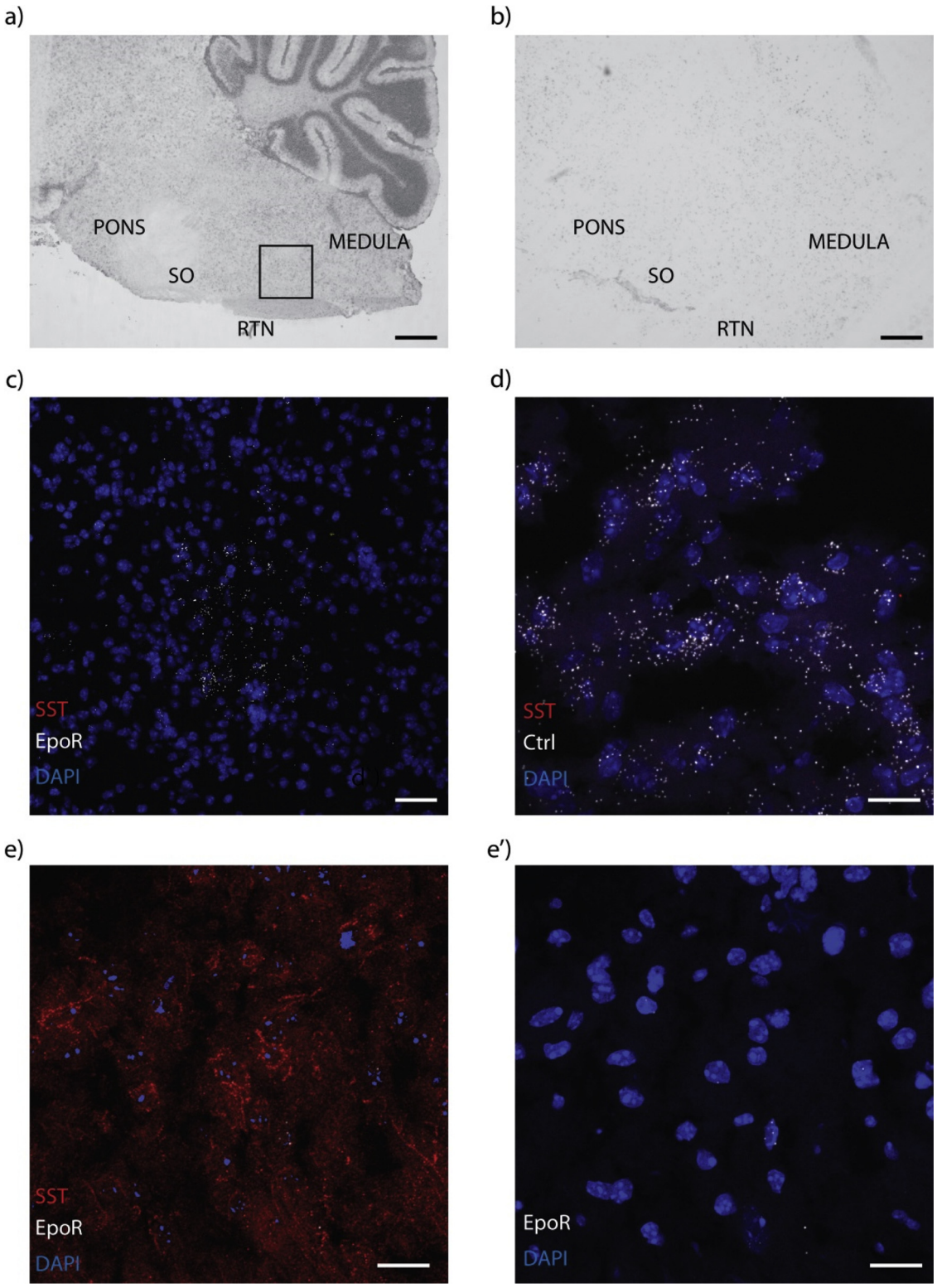

Fig. 4. Expression of EPOR mRNA in mouse brainstems evaluated by the fluorescence *in-situ* hybridization technique (FISH) combined with immunofluorescence labeling of somatostatin cells at age P25. Expression of EPOR mRNA is present in brainstem sections from the respiratory control centers (RTN, pBötC, and nucleus ambiguous;NA) at multiple rostrocaudal sections but not on SST positive cells. Total sagittal section of the brainstem with antisense probe (a) and sense probe as control (b), showing the area (inset square) in which EpoR and SST was analyzed (c and e). (c) EPOR mRNA expression in SST negative cells. (d) FISH-positive control with a probe that targets species-specific housekeeping genes. (e) EPOR mRNA expression in SST positive cells. Scale bar: A and B, 500 μm; C: 50 μm and D, E: 20 μm.

7. Summary

In conclusion, this short review summarizes the role of EPO in the neural control of ventilation and provides new data about the distribution of the classical EPOR in specific brainstem nuclei associated with central chemosensitivity and control of ventilation in the ventrolateral medulla. We show that EPOR's are mainly on somatostatin negative cells, leading us to conclude that EPO signaling may act

through its classical EPOR on glutamatergic neuron populations that are the primary source of rhythmic inspiratory excitatory drive. This work underlies the importance of EPO signaling in the central control of ventilation across development and adulthood in normoxic and hypoxic conditions and provides new insights on the cellular localization of the receptors and consequently the respiratory control mechanism by which EPO may act.

Acknowledgments

The authors wish to thank the Canadian Institutes of Health Research (CIHR). Catalyst Grant: Sex as a Variable in Biomedical Research (SVB-158607). The salary of Jorge Soliz is supported by the“Fonds de recherche du Quebec-Sante” (FRQ-S; FQ121919). The authors have no actual or potential conflict of interest to disclose. The authors wish to thank to Dr. Tara Janes for her appraisal of the manuscript and editorial assistance.

References

Al-Qahtani, J.M., Abdel-Wahab, B.A., Abd El-Aziz, S.M., 2014. Long-term moderate dose exogenous erythropoietin treatment protects from intermittent hypoxia-induced spatial learning deficits and hippocampal oxidative stress in young rats. Neurochem. Res. 39, 161–171.

Alnaeeli, M., Wang, L., Piknova, B., Rogers, H., Li, X., Noguchi, C.T., 2012. Erythropoietin in brain development and beyond. Anat. Res. Int. 2012, 953264.

Ballot, O., Joseph, V., Soliz, J., 2015. Endogenous brain erythropoietin is a potent sex-specific respiratory stimulant in adult and newborn mice. J. Appl. Physiol. 1985 jap 00143 02015.

Bauer, C., 1995. Erythropoietin—from gene structure to therapeutic applications. J. Perinat. Med. 23, 77–81.

Bernaudin, M., Marti, H.H., Roussel, S., Divoux, D., Nouvelot, A., MacKenzie, E.T., Petit, E., 1999. A potential role for erythropoietin in focal permanent cerebral ischemia in mice. J. Cereb. Blood Flow Metab. 19, 643–651.

Beschin, A., Bilej, M., Torreele, E., De Baetselier, P., 2001. On the existence of cytokines in invertebrates. Cell. Mol. Life Sci. 58, 801–814.

Bissonnette, J.M., 2000. Mechanisms regulating hypoxic respiratory depression during fetal and postnatal life. Am. J. Physiol. Regul. Integr. Comp. Physiol. 278, R1391–1400.

Byts, N., Siren, A.L., 2009. Erythropoietin: a multimodal neuroprotective agent. Exp. Transl. Stroke Med. 1, 4.

Caravagna, C., Kinkead, R., Soliz, J., 2014. Post-natal hypoxic activity of the central respiratory command is improved in transgenic mice overexpressing Epo in the brain. Respir. Physiol. Neurobiol. 200, 64–71.

Caravagna, C., Schneider-Gasser, E.M., Ballot, O., Joseph, V., Soliz, J., 2015. Chronic overexpression of cerebral Epo improves the ventilatory response to acute hypoxia during the postnatal development. Int. J. Dev. Neurosci.

Caravagna, C., Soliz, J., 2015. PI3K and MEK1/2 molecular pathways are involved in the erythropoietin-mediated regulation of the central respiratory command. Respir. Physiol. Neurobiol. 206, 36–40.

Carroll, J.L., Agarwal, A., 2010. Development of ventilatory control in infants. Paediatr. Respir. Rev. 11, 199–207.

Dayyat, E.A., Zhang, S.X., Wang, Y., Cheng, Z.J., Gozal, D., 2012. Exogenous erythropoietin administration attenuates intermittent hypoxia-induced cognitive deficits in a murine model of sleep apnea. BMC Neurosci. 13, 77.

de Lima, M.N., Polydoro, M., Laranja, D.C., Bonatto, F., Bromberg, E., Moreira, J.C., Dal-Pizzol, F., Schroder, N., 2005. Recognition memory impairment and brain oxidative stress induced by postnatal iron administration. Eur. J. Neurosci. 21, 2521–2528.

Di Fiore, J.M., Martin, R.J., Gauda, E.B., 2013. Apnea of prematurity—perfect storm. Respir. Physiol. Neurobiol. 189, 213–222.

Di Fiore, J.M., Poets, C.F., Gauda, E., Martin, R.J., MacFarlane, P., 2016. Cardiorespiratory events in preterm infants: interventions and consequences. J. Perinatol. 36, 251–258.

Digicaylioglu, M., Bichet, S., Marti, H.H., Wenger, R.H., Rivas, L.A., 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.

Ehrenreich, H., Hasselblatt, M., Knerlich, F., von Ahsen, N., Jacob, S., Sperling, S., Woldt, H., Vehmeyer, K., Nave, K.A., Siren, A.L., 2005. A hematopoietic growth factor, thrombopoietin, has a proapoptotic role in the brain. Proc. Natl. Acad. Sci. U. S. A. 102, 862–867.

Elliot-Portal, E., Laouafa, S., Arias, C., Janes, T.A., Joseph, V., Soliz, J., 2018. Cerebral erythropoietin prevents cardio-respiratory dysfunction induced by chronic intermittent hypoxia. Sleep in press.

Elliott, S., Busse, L., Bass, M.B., Lu, H., Sarosi, I., Sinclair, A.M., Spahr, C., Um, M., Van, G., Begley, C.G., 2006. Anti-Epo receptor antibodies do not predict Epo receptor expression. Blood 107, 1892–1895.

Elliott, S., Busse, L., McCaffery, I., Rossi, J., Sinclair, A., Spahr, C., Swift, S., Begley, C.G., 2010. Identification of a sensitive anti-erythropoietin receptor monoclonal antibody allows detection of low levels of EpoR in cells. J. Immunol. Methods 352, 126–139.

Erenberg, A., Leff, R.D., Haack, D.G., Mosdell, K.W., Hicks, G.M., Wynne, B.A., 2000. Caffeine citrate for the treatment of apnea of prematurity: a double-blind, placebo-controlled study. Pharmacotherapy 20, 644–652.

Filiano, J., Kinney, H., 1994. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. Biol. Neonate 65, 194–197.

Gassmann, M., Heinicke, K., Soliz, J., Ogunshola, O.O., 2003. Non-erythroid functions of erythropoietin. Adv. Exp. Med. Biol. 543, 323–330.

Hahn, N., Knorr, D.Y., Liebig, J., Wustefeld, L., Peters, K., Buscher, M., Bucher, G., Ehrenreich, H., Heinrich, R., 2017. The insect ortholog of the human orphan cytokine receptor CRLF3 is a neuroprotective erythropoietin receptor. Front. Mol. Neurosci. 10, 223.

Heinrich, R., Gunther, V., Miljus, N., 2017. Erythropoietin-Mediated Neuroprotection in Insects Suggests a Prevertebrate Evolution of Erythropoietin-Like Signaling. Vitam. Horm. 105, 181–196.

Jelkmann, 1994a. Biology of erythropoietin. Clin. Investig. 72, 3–10.

Jelkmann, W., 1994b. Biology of erythropoietin. Clin. Investig. 72, S3–10.

Jobe, A.H., Kallapur, S.G., 2010. Long term consequences of oxygen therapy in the neonatal period. Semin. Fetal Neonatal Med. 15, 230–235.

Joseph, V., Doan, V.D., Morency, C.E., Lajeunesse, Y., Bairam, A., 2006. Expression of sex-steroid receptors and steroidogenic enzymes in the carotid body of adult and newborn male rats. Brain Res. 1073-1074, 71–82.

Juul, S.E., Anderson, D.K., Li, Y., Christensen, R.D., 1998a. Erythropoietin and erythropoietin receptor in the developing human central nervous system. Pediatr. Res. 43, 40–49.

Juul, S.E., Harcum, J., Li, Y., Christensen, R.D., 1997. Erythropoietin is present in the cerebrospinal fluid of neonates. J. Pediatr. 130, 428–430.

Juul, S.E., Yachnis, A.T., Christensen, R.D., 1998b. Tissue distribution of erythropoietin and erythropoietin receptor in the developing human fetus. Early Hum. Dev. 52, 235–249.

Kirkeby, A., van Beek, J., Nielsen, J., Leist, M., Helboe, L., 2007. Functional and immunochemical characterisation of different antibodies against the erythropoietin receptor. J. Neurosci. Methods 164, 50–58.

Knabe, W., Knerlich, F., Washausen, S., Kietzmann, T., Siren, A.L., Brunnett, G., Kuhn, H.J., Ehrenreich, H., 2004. Expression patterns of erythropoietin and its receptor in the developing midbrain. Anat. Embryol. (Berl.) 207, 503–512.

Kumral, A., Genc, S., Kutluk, K., Ozkan, H., 2010. Safety concerns with the clinical use of erythropoietin in acute ischemic stroke. Stroke 41, e469.

Kumral, A., Tuzun, F., Oner, M.G., Genc, S., Duman, N., Ozkan, H., 2011. Erythropoietin in neonatal brain protection: the past, the present and the future. Brain Dev. 632–643.

Lagranha, C.J., Silva, T.L.A., Silva, S.C.A., Braz, G.R.F., da Silva, A.I., Fernandes, M.P., Sellitti, D.F., 2017. Protective effects of estrogen against cardiovascular disease mediated via oxidative stress in the brain. Life Sci.

Laouafa, S., Iturri, P., Arias-Reyes, C., Marcouiller, F., Gonzales, M., Joseph, V., Bairam, A., Soliz, J., 2019. Erythropoietin and caffeine exert similar protective impact against neonatal intermittent hypoxia: apnea of prematurity and sex dimorphism. Exp. Neurol in press.

Liu, Z.Y., Chin, K., Noguchi, C.T., 1994. Tissue specific expression of human erythropoietin receptor in transgenic mice. Dev. Biol. 166, 159–169.

Marti, H.H., 2003. Function of Erythropoietin in the Brain. Johonson City, TN USA. .

Masuda, S., Nagao, M., Takahata, K., Konishi, Y., Gallyas Jr, F., Tabira, T., Sasaki, R., 1993. Functional erythropoietin receptor of the cells with neural characteristics. Comparison with receptor properties of erythroid cells. J. Biol. Chem. 268, 11208–11216.

Miljus, N., Heibeck, S., Jarrar, M., Micke, M., Ostrowski, D., Ehrenreich, H., Heinrich, R., 2014. Erythropoietin-mediated protection of insect brain neurons involves JAK and STAT but not PI3K transduction pathways. Neuroscience 258, 218–227.

Miljus, N., Massih, B., Weis, M.A., Rison, J.V., Bonnas, C.B., Sillaber, I., Ehrenreich, H., Geurten, B.R., Heinrich, R., 2017. Neuroprotection and endocytosis: erythropoietin receptors in insect nervous systems. J. Neurochem. 141, 63–74.

Millet, A., Bouzat, P., Trouve Buisson, T., Batandier, C., Pernet Gallay, K., Gaide Chevronnay, L., Barbier, E., Debillon, T., Fontaine, E., Payen, J.F., 2015. Erythropoietin and its derivates modulate mitochondrial dysfunction after diffuse traumatic brain injury. J. Neurotrauma.

Oshima, N., Onimaru, H., Yamagata, A., Itoh, S., Matsubara, H., Imakiire, T., Nishida, Y., Kumagai, H., 2018. Erythropoietin, a putative neurotransmitter during hypoxia, is produced in RVLM neurons and activates them in neonatal Wistar rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 314, R700–R708.

Ostrowski, D., Ehrenreich, H., Heinrich, R., 2011. Erythropoietin promotes survival and regeneration of insect neurons in vivo and in vitro. Neuroscience 188, 95–108.

Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., Buga, A.M., 2013. ROS and brain diseases: the good, the bad, and the ugly. Oxid. Med. Cell. Longev. 2013, 963520.

Seaborn, T., Isidoro, M.G., Villalpando, G., Grenacher, B., Soria, R., Soliz, J., 2011. Enhanced erythropoietin expression in the brainstem of newborn rats at high altitude. Neurosci. Lett. 502, 33–36.

Siren, A.L., Ehrenreich, H., 2001. Erythropoietin—a novel concept for neuroprotection. Eur. Arch. Psychiatry Clin. Neurosci. 251, 179–184.

Soliz, J., Gassmann, M., Joseph, V., 2007a. Downregulation of soluble erythropoietin receptor in the mouse brain is required for the ventilatory acclimatization to hypoxia. J. Physiol. 583 (1), 329–336.

Soliz, J., Joseph, V., 2005. Perinatal steroid exposure and respiratory control during early postnatal life. Respir. Physiol. Neurobiol. 149, 111–122.

Soliz, J., Joseph, V., Ogunshola, O., Gassmann, M., 2006. Erythropoietin controls ventilation in hypoxia. Physiol. News 63, 2.

Soliz, J., Joseph, V., Soulage, C., Becskei, C., Vogel, J., Pequignot, J.M., Ogunshola, O., Gassmann, M., 2005. Erythropoietin regulates hypoxic ventilation in mice by interacting with brainstem and carotid bodies. J. Physiol. 568, 559–571.

Soliz, J., Soulage, C., Hermann, D.M., Gassmann, M., 2007b. Acute and chronic exposure to hypoxia alters ventilatory pattern but not minute ventilation of mice over-expressing erythropoietin. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, R1702–R1710.

Suzuki, N., Ohneda, O., Takahashi, S., Higuchi, M., Mukai, H.Y., Nakahata, T., Imagawa, S., Yamamoto, M., 2002. Erythroid-specific expression of the erythropoietin receptor rescued its null mutant mice from lethality. Blood 100, 2279–2288.

Viemari, J., Burnet, H., Bévengut, M., Hilaire, G., 2003. Perinatal maturation of the mouse respiratory rhythm-generator: in vivo and in vitro studies. Eur. J. Neurosci. 17, 1233–1244.

Wenger, R., Marti, H., Bauer, C., Gassmann, M., 1998. Optimal erythropoietin expression in human hepatoma cell lines requires activation of multiple signalling pathways. Int. J. Mol. Med. 2, 317–324.

Wyder, S., Kriventseva, E.V., Schröder, R., Kadowaki, T., Zdobnov, E.M., 2007. Quantification of ortholog losses in insects and vertebrates. Genome Biol. 8, R242.