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Review

Erythropoietin and respiratory control at adulthood and during early postnatal life☆

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ABSTRACT

Erythropoietin (Epo) was originally discovered as a cytokine able to increase the production of red blood cells upon conditions of reduced oxygen availability. Now we know that Epo does far more than "only" augmenting the number of erythrocytes. Since the demonstration that Epo (and its receptor) is expressed in the mammalian brain, several elegant experiments were performed to reveal the function of this molecule in the neuronal tissue. Accordingly to its anti-apoptotic, neurotrophic and proliferative effects in the bone marrow, it was suitably suggested that upon pathological conditions Epo exerts neuroprotective functions (i.e. reducing the infarct volume of stroke, thus allowing better and faster recovery). We considered however, that Epo in brain might also exert a physiological function. Indeed, we found that Epo is an important modulator of the respiratory control system. By using adult mice we showed that Epo increases the hypoxic ventilatory response by interacting with both the central respiratory network (brainstem) as well as the main peripheral sensory organs detecting systemic hypoxia, the carotid bodies. More recently, our research turned to examine the exciting hypothesis that Epo is also implicated in the regulation of the neuronal control of ventilation during the postnatal development. The objective of this review is to summarize the role and mode of action of Epo on respiratory control in adult mammals and highlight the potential pathways by which this cytokine achieve this function. Additionally, we review recent evidences showing that Epo play a crucial role in setting the respiratory motor output (measured on the isolated brainstem spinal cord preparation, en bloc technique) during the early postnatal life.

1. Introduction

Although often been treated as a "classic endocrine hormone" erythropoietin (Epo) is also a cytokine that fulfills several functions other than simply increasing red blood cell number. Furthermore, it has become clear over the last decade that the kidney is far from being the sole organ producing Epo and that this cytokine, especially in the brain, has tissue-protective functions. Proof-of-concept trials performed in adult and newborn patients have revealed that cerebral Epo (i.e. Epo synthesized in neurons and astrocytes) exerts crucial protective functions against hyperoxia, hypoxia, and ischemia-induced damage (Kumral et al., 2011). In fact, recombinant human Epo is now one of the most promising neuroprotective agents under investigation (Digicaylioglu, 2010). In line with these observations, the number of scientific publications on the potential, specific use of Epo in neonates has tripled in the last 10 years. Advances in this topic have established that (i) the Epo receptor (EpoR) is expressed extensively in mouse (Knabe et al., 2004) and human (Juul et al., 1998) brain fetuses at early gestation days (1 week and 5 weeks, respectively) and remains thereupon expressed (albeit with modulated variations) throughout life, and (ii) systemic Epo injection improves the neurodevelopmental outcome of extremely preterm infants (Neubauer et al., 2010). Importantly, neuroprotection requires the use of high-dose Epo (1000–30,000 U/kg). However, such dosage is well above the range used to treat anemia (500 U/kg) and has prompted concerns about potential adverse effects of Epo. Whereas long-term Epo treatment in adults has been associated with hypertension, seizures, thrombotic events and polycythemia, interestingly, repeated high-dose Epo treatment in infants and neonates has no discernible adverse effects (Kellert et al., 2007; Kumral et al., 2011).

Regarding the effect of Epo on respiration, subcutaneous treatment of premature neonates (gestational age < 30 weeks) with Epo (300 U/kg/dose, 3 times/week) has been recently reported to improve both erythropoiesis (higher hemoglobin, hematocrit and reticulocytes) and ventilatory function (reduced need of assisted ventilation and O_2 supplementation) (Tempera et al., 2011).

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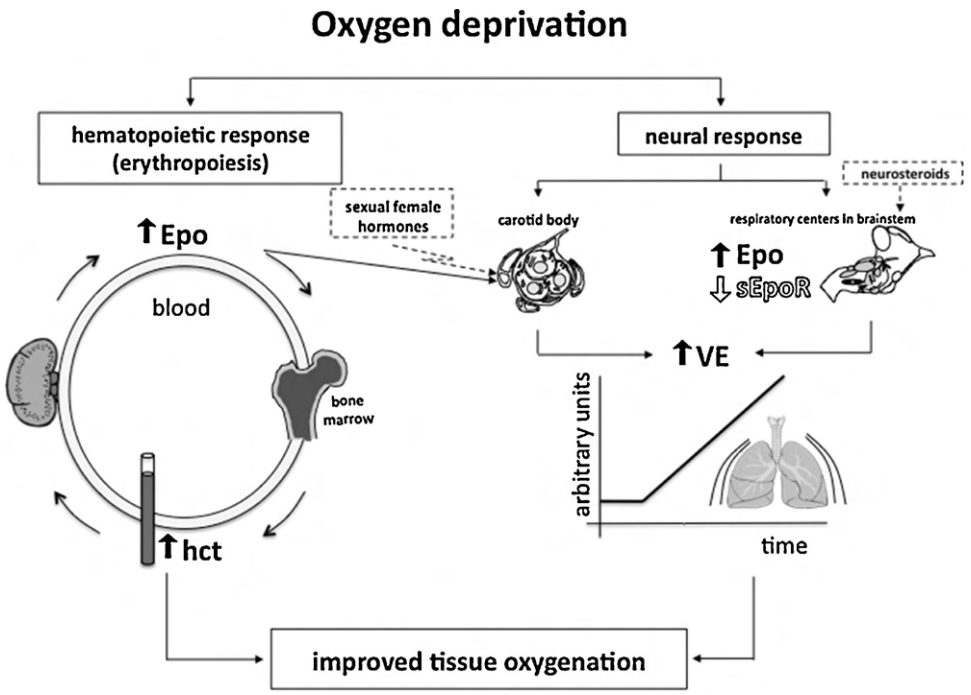

Fig. 1. Model of ventilatory response to hypoxia showing the contribution of cerebral and plasma Epo. Carotid bodies sense the drop of arterial oxygen pressure during the first minutes of hypoxia, thus leading to a fast response to hypoxia. Persistent exposure to hypoxia promotes a higher secretion of Epo by the kidney. Higher level of plasma Epo augments the oxygen carrying capacity (by gradual increase of the hematocrit: hct), but also contributes to the regulation of ventilation (VE) through regulating the activity of the carotid bodies. In parallel, the level of cerebral Epo is increased in brainstem while the level of sEpoR is decreased, thus contributing to the regulation of central ventilation.

Adapted from Soliz et al. (2007a).

Although a better hematological status may help to stabilize respiration, the remarkable improvement of ventilatory function had not been reported in studies where only blood transfusions were applied (Kasat et al., 2011). Indeed, we have shown that cerebral Epo prevents hypoxic-induced respiratory depression in adult mice. By using adult transgenic mice overexpressing Epo in brain only (Tg21), or in brain and plasma (Tg6), we showed that cerebral Epo activates the central respiratory network by the modulation of catecholamine synthesis in the brainstem (Soliz et al., 2005, 2007a). In addition, in adult wild type mice and humans we demonstrated that plasma Epo (i.e. Epo synthesized in the kidney) increases the hypoxic ventilatory response via stimulation of the carotid bodies (the main peripheral sensory organs detecting systemic hypoxia) (Soliz et al., 2005, 2007b, 2009). Fig. 1 shows a schematic diagram summarizing these findings.

Obviously, these results provide a strong rationale for exploring the implication of Epo in the development of the neural respiratory control system. Accordingly, the last efforts of our research were directed to this important and unexplored aspect of the non-erythropoietic, physiological function of Epo. This review summarizes briefly our most recent findings and, at the same time underlines the important aspects that still need to be investigated. The reader should keep in mind that our findings certainly represent the emerging tip of a dense iceberg. However, our current data clearly show that Epo plays an important role on respiratory control during early life, which is overall an encouraging thought.

2. Epo, its receptor and its endogenous antagonist

Epo is a 165-aa glycoprotein (34 kDa) that belongs to the type I cytokine superfamily and plays an essential role in erythropoiesis (Jelkmann, 2007). Epo is mainly produced in the kidney, but in the last decade, Epo has been shown to be expressed in several other tissues, including the brain (Marti et al., 1996). Epo expression is low under normal oxygenation, but under hypoxic condition, Epo mRNA (Marti et al., 1996) and protein (Chikuma et al., 2000) accumulate 5- to 20-fold in the brain and 3- to 200-fold in the kidney. Epo expression is mainly regulated by the hypoxia-inducible factor (Gassmann et al., 2000), and exerts its biological effects by binding to its specific receptor. EpoR is expressed in the kidney, but is also widely distributed in the mammalian brain (Genc et al., 2004). In fetal mouse, EpoR 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). As any other cytokine receptor, EpoR is also synthesized in a truncated, soluble form (sEpoR), which competes with the functional receptor to bind Epo, thus acting as an endogenous antagonist (Westphal et al., 2002). Synthesis of sEpoR occurs by alternative splicing of EpoR mRNA (Westphal et al., 2002). The resulting short sEpoR polypeptide is made of the sole extracellular EpoR domain and is therefore secreted into the extracellular milieu in every tissue expressing the EpoR (Westphal et al., 2002). In the developing mouse, EpoR localizes in the neural tube together with proliferating neuroprecursors (Alnaeeli et al., 2012). EpoR level in the neural tube at embryonic day 10.5 is comparable to that found in adult hematopoietic tissue. Brain expression then decreases >1000-fold during development, reaching low levels at birth (about 0.2 mU/ml), which persists until adulthood (Juul et al., 1998). Epo and EpoR have been detected in several brain regions in rodents and primates, including cortex, hippocampus, amygdala, cerebellum, hypothalamus, and caudate nucleus (Siren and Ehrenreich, 2001). In adult mice, we have shown that EpoR is expressed in the main brainstem areas associated with respiratory control, such as the pre-Bötzinger complex, motor neurons, trapezoid body and nucleus tractus solitarius and catecholaminergic nuclei (Soliz et al., 2005). In addition, we found

that sEpoR is also expressed in adult mouse brain and that its expression is downregulated upon chronic hypoxia (Soliz et al., 2007a).

3. Non-erythropoietic, neuroprotective function of Epo in the brain

Epo has been shown to play a critical role in the development, maintenance, protection and repair of the nervous system (Kumral et al., 2011). Epo is involved in neuroprotection, neurogenesis and angiogenesis, and plays an important role as a neurotrophic factor (Rabie and Marti, 2008). Studies in adult rodents, monkeys and humans show that Epo exerts a protective function in several ischemic stroke models, traumatic brain injury, spinal cord injury and perinatal asphyxia (Rabie and Marti, 2008). Remarkably, recent studies demonstrate that Epo also protects against chronic neurodegenerative diseases and mental disorders (e.g. multiple sclerosis, Sattler et al., 2004; epilepsy, Kondo et al., 2009; schizophrenia, Ehrenreich et al., 2004; Alzheimer's, Assaraf et al., 2007), modulates cognitive processing (Miskowiak et al., 2010), improves mood (Miskowiak et al., 2009) and protects against amygdala-dependent tone fear conditioning (Miu et al., 2004).

The effect of Epo in neuroprotection has also been demonstrated by repeated administration of high doses of Epo in newborn animals and preterm infants (Neubauer et al., 2010). Epo has also been identified in brain and cerebrospinal fluid following neonatal brain injuries such as asphyxia (Sola et al., 2005). Indeed, Epo acts as a neuroprotective agent against hypoxia, since infusion of sEpoR (which competes with EpoR to bind Epo and bloc its function) into the brain results in neuronal death in the hippocampus (Xiong et al., 2011). Under hypoxia–ischemia conditions (produced by permanent carotid artery ligation in 7-d-old rats), increased Epo immunoreactivity was observed, but a remarkable upregulation of EpoR was also evidenced at earlier times, thus suggesting that a rapid response in EpoR expression precedes Epo production (Spandou et al., 2004). In addition, Epo treatment in 7-d-old rats subjected to hypoxia–ischemia led to enhanced revascularization, decreased infarct volume, improved neurological outcomes, increased neurogenesis in the subventricular zone and increased migration of neuronal progenitors into ischemic cortex (Alnaeeli et al., 2012).

In human, a recent study on preterm birth showed that postnatal Epo treatment improves the neurodevelopmental outcome of 148 extremely preterm infants (Neubauer et al., 2010). Over a period of 10–13 years, it was observed that the school outcome in the Epo group was significantly better than in untreated children (Neubauer et al., 2010).

The mechanisms by which Epo exerts its neuroprotective functions include the inhibition of apoptosis by sustaining the expression of Bcl-2 and Bcl-x_L proteins, the prevention of glutamate-induced cell death, the modulation of neuronal activity and neurotransmitter release by inducing variations in intracellular Ca^{2+} concentration in neurons, an upregulation of antioxidative enzymes, a decrease in inflammation, and the promotion of growth, differentiation and function of dopaminergic cells (Alnaeeli et al., 2012; Rabie and Marti, 2008; Siren and Ehrenreich, 2001). Importantly, Epo is able to cross the blood–brain-barrier at high doses (Banks et al., 2004).

4. Non-erythropoietic, physiological function of Epo in the brain

Apart from a neuroprotective function in pathological events, we postulated that Epo has also an important physiological role upon hypoxic conditions. Epo, at adulthood, modulates the ventilatory response when oxygen supply is lowered. Note that physiological hypoxia occurs at high altitude. However, sustained hypoxemic exposure, as experienced by patients with diseases including emphysema, chronic bronchitis, cystic fibrosis or sleep apnoeas, can result in significant cardiovascular sequalae such as pulmonary hypertension (Naeije and Barbera, 2001), right heart failure (Naeije, 2005), coronary heart disease (Mooe et al., 2000) and systemic hypertension (Nieto et al., 2000). During sustained hypoxia, Epo synthesis in the kidney is accelerated, resulting in increased plasma Epo levels (Jelkmann, 2007). Moreover, sustained hypoxia promotes the increase of Epo in brain tissue (Digicaylioglu et al., 1995). It is the effect of brain-derived Epo overexpression that we studied in the neuronal control of respiration of adult rodents. Our main findings are described below.

4.1. Epo and the central respiratory network at adulthood

To study the impact of Epo in the neural control of respiration, we evaluated the respiratory activity (by plethysmography) of wild type and transgenic mice overexpressing Epo in brain only (Tg21) (Soliz et al., 2005). Tg21 animals represent a suitable animal model for these studies because, despite constitutively overexpressing Epo in brain, they show normal Epo plasma levels, thus having normal levels of hemoglobin and hematocrit. Our results showed significantly higher ventilatory response to hypoxia in Tg21 mice compared to control wild type (Soliz et al., 2005). Bilateral transection of carotid sinus nerve (chemodenervation) is a common approach in studying the oxygen sensing process in brainstem in the absence of peripheral (carotid body) interference (Pascual et al., 2004). Accordingly, we compared the ventilatory response to acute hypoxia of chemodenervated wild type and Tg21 animals. Interestingly, while wild type mice showed life-threatening apneas, Tg21 animals maintained sustained ventilation. This data indicates that Epo overexpression in the brainstem allows maintenance of high respiratory activity during acute hypoxia despite the lack of information from the peripheral chemoreceptors (Soliz et al., 2005, 2006).

In a next step, we hypothesized that ventilatory acclimatization to chronic hypoxia (3 days at 10% O_2) in adult mice would also be facilitated in Tg21 mice. Indeed, compared to wild type animals, Tg21 mice showed enhanced ventilatory response to chronic hypoxia (Soliz et al., 2005). Moreover, in an attempt to verify our data by using WT mice, we focused the sEpoR (the endogenous antagonist of Epo). First, we observed that chronic hypoxia produces a drastic downregulation of the sEpoR in the central nervous system of WT mice (Soliz et al., 2007b). In a following step, when sEpoR was chronically infused in the nervous system of mice by a minipump, the process of ventilatory acclimatization to chronic hypoxia (defined as a gradual increase in ventilation to compensate for the low O_2 availability) was abolished (Fig. 2). In parallel, the neural Epo concentration was decreased by 50%. These results show that the neural regulation of Epo and its antagonist sEpoR play a critical role in the central nervous system in stabilizing the ventilatory activity and thus ensuring the systemic oxygen delivery under low O_2 conditions (Soliz et al., 2007b). In line with these findings, immunohistochemical analysis revealed that brainstem EpoR is expressed in neurons involved in respiratory rhythmogenesis (i.e. the pre-Bötzinger complex), sensory integration (nucleus tractus solitarius) and in catecholaminergic neural groups (A6 and A5 in the pons; A2/C2 and A1/C1 in the medulla oblongata) (Soliz et al., 2005).

Concerning mechanisms, obviously it rises the question whether Epo modulates the neural ventilation by activating neuroprotective pathways. In neural-glial cell cultures, Epo provides neuroprotection action by activating the Janus-tyrosine kinase 2 (JAK-2) (Rabie and Marti, 2008). Subsequently,

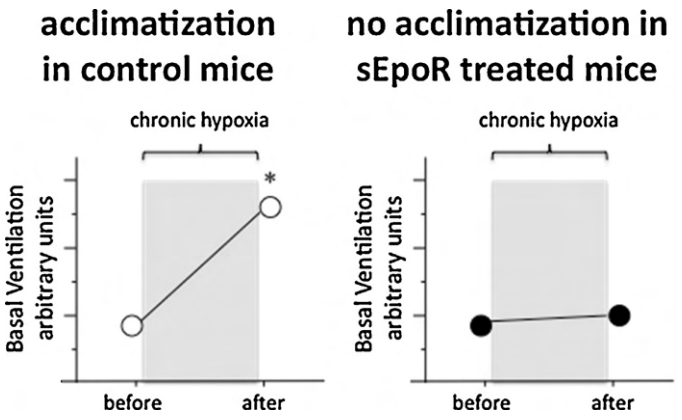

Fig. 2. Intracerebral infusion of sEpoR abolishes the ventilatory acclimatization to chronic hypoxia. Normoxic minute ventilation was evaluated before and after animals were exposed to chronic hypoxia of 10% O_2 during 3 days. After acclimatization, control animals showed large increase of normoxic basal ventilation. In contrast, this elevation was abolished in sEpoR-treated mice.
Adapted from Soliz et al. (2007a).

JAK-2 phosphorylates several neuroprotective pathways such as the MAP kinases, ERK-1/-2, PI3 kinase/Akt, JNK, and signal transducers and activators of transcription (STAT)-5 (Rabie and Marti, 2008). Accordingly, we performed western blot analysis to determine whether the Epo signal transduction in the brainstem of tg21 mice was prolonged in comparison to WT animals. Interestingly, no differences of neural JAK-2 or associated pathways between tg21 and WT mice were obtained. These results suggested that the Epo-mediated increased hypoxic ventilation in tg21 mice is not related to higher activation of neuroprotective signal transduction (Gassmann et al., 2009). However, important for our work was to learn that Epo has also been recognized as a factor able to modulate release of catecholamines (Soliz et al., 2005, 2007b). Since catecholamines in brainstem are central molecules in the modulation of ventilation upon hypoxic conditions (Hilaire et al., 2004), we evaluated the noradrenaline content and the tyrosine hydroxylase (TH) activity in tg21 and WT mice. Compared to WT controls, tg21 mice show altered catecholaminergic content in brainstem, higher levels in pons, but lower levels in medulla. These data are in line with the report showing that increased hypoxic ventilation is associated with higher catecholamine level in pontial A5 cell group (Dick and Coles, 2000) and with lower catecholamine level in the medullary A1C1 and A2C2 cell groups (Champagnat et al., 1979). Thus, our results suggest that higher Epo level modulates the catecholamine synthesis in brainstem. Once hypoxic, this alteration affects the ventilatory response by increasing the respiratory frequency.

4.2. Epo and the central respiratory network during the postnatal development

During perinatal life, the respiratory control system undergoes intense development and is highly responsive to stimuli emerging from the environment, which modulate the respiratory motor output. Around birth, the hypoxic ventilatory response is biphasic, with a rapid increase of minute ventilation followed by a drop below resting values, while mature HVR is characterized by sustained hyperpnea throughout the hypoxic exposure. To date the implication of Epo in the respiratory control network of newborns remain poorly studied. Preliminary studies obtained in our laboratory demonstrate that in neonatal mice, Epo stimulates the core brainstem network controlling respiration. By using in vitro "en bloc" electrophysiological technique, we recorded the fictive breathing produced by brainstem-spinal cord preparations of 4-d-old mice. Prior to experimentation, preparations were incubated with

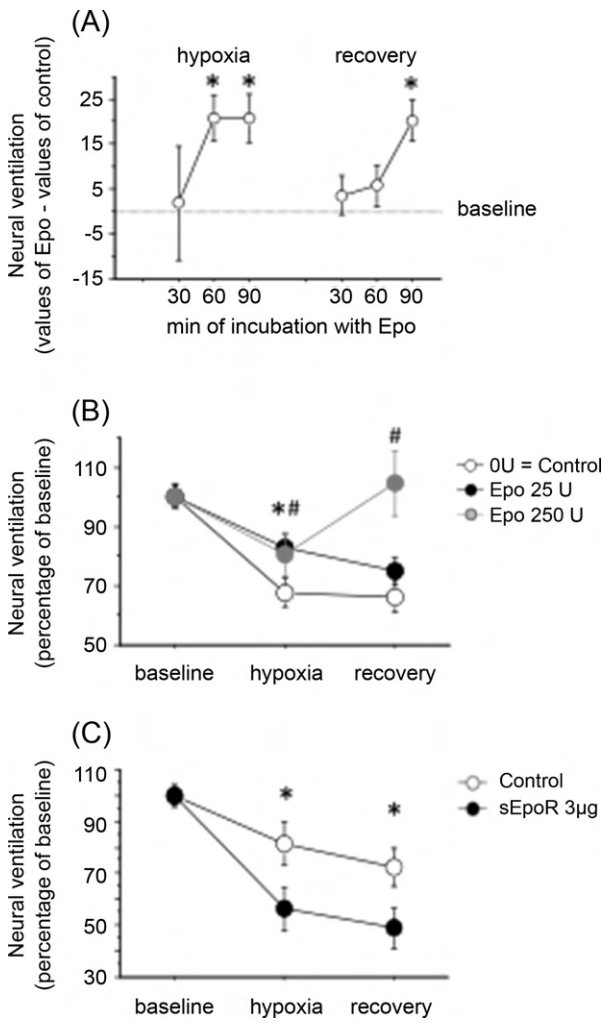

Fig. 3. In vitro electrophysiology (en bloc) in brainstem-spinal cord preparations of mice at postnatal day 4 (n = 8–10/group). Each point represents the mean of 10 min registration under the specified condition. Preparations were superfused with artificial cerebrospinal fluid (aCSF), bubbled with 95%O_2, 5%CO_2 (baseline/recovery), or 95%N_2, 5%CO_2 (hypoxia). Neural ventilation = frequency × amplitude. (A) Time-dependent impact of pre-incubation with 25 U Epo (*p < 0.05 Epo vs baseline). (B) Impact of pre-incubation with different doses of Epo during 60 min (*p < 0.05 Epo 25 U vs control; #p < 0.05 Epo 250 U vs control). (C) Impact of pre-incubation with sEpoR (Epo antagonist) during 60 min (*p < 0.05 sEpoR vs control).
Adapted from Khemiri et al. (2012).

Epo or vehicle. Burst activity of the 4th cervical ventral root was next recorded under normoxic, hypoxic and recovery conditions. Our findings show that Epo prevents the hypoxic depression, and produces an increase during the recovery phase in a time- and dose-dependent manner. In addition, we found that incubation with sEpoR decreases ventilation upon hypoxia and during the recovery phase, implying that endogenous Epo regulates the brainstem motor output in response to hypoxia (Fig. 3) (Khemiri et al., 2012).

These encouraging findings raise a number of questions regarding the role of Epo in the neural control of ventilation at sea level, but also in the acclimatization/adaptation to high altitude. Accordingly, we recently evaluated the postnatal ontogeny of cerebral Epo concentration in Sprague–Dawley rats permanently living and reproducing at high altitude (3600 m in La Paz, Bolivia). These results show that in high-altitude rats, postnatal Epo concentration is higher in the brainstem than in the forebrain. Moreover, although Epo concentrations in the forebrain of high-altitude rats and sea-level controls are similar, Epo level in the brainstem is, quite surprisingly, 2-fold higher in high-altitude rats than in

sea-level controls (Seaborn et al., 2011). Altogether, these findings strongly suggest that Epo plays a key role in postnatal development and maturation of the brainstem respiratory network, as well as in the ability to tolerate physiological (e.g. high altitude) and pathological (e.g. respiratory disorders) levels of O_2 deprivation.

5. Non-erythropoietic, physiological function of Epo in the carotid body

In 1930, after the classical studies of Heymans (1945), the carotid body has been accepted as a chemoreceptor that monitors the oxygen tension of systemic arterial blood. Almost at this early time, Epo was already associated with the chemosensory organ. Since it was observed in rabbits that the removal of both carotid bodies gives rise to an altered blood picture, the question was whether the carotid sinus participates in the regeneration of blood (Hansen et al., 1973). Nevertheless, this was not confirmed when chemodenervated cats showed a more vigorous erythropoietin response to hypoxia (Gillis and Mitchell, 1973). No relationship between Epo and carotid bodies was mentioned in the following 20 years until when Sasaki and his co-workers reported the presence of functional Epo receptor in PC12 cells (Sasaki et al., 2001). Later, in a beautiful work, Koshimura and co-workers showed that Epo in PC12 cells is able to induce membrane depolarization, increase the intracellular calcium concentration, increase the tyrosine hydroxylase activity and the dopamine biosynthesis, to augment the production of nitric oxide and to promote the cell survival (Koshimura et al., 1999). Based on this work, we hypothesized that peripheral chemoreceptors might also express Epo receptors and that circulatory Epo participates in the modulation of the carotid body-mediated hypoxic ventilatory response

5.1. Epo and carotid bodies at adulthood

Increasing ventilation is the most important hypoxic response when mammals are acutely or chronically exposed to high altitude (Joseph and Pequignot, 2009; Joseph et al., 2002). Accordingly, we hypothesized that a higher concentration of circulating Epo stimulates the ventilatory response to hypoxia by interacting with the carotid bodies. Therefore, our first step was performing immunostaining in serial lateral sections of the carotid body bifurcation. Tyrosine hydroxylase (TH) was used to identify the glomus cells. As predicted, a dense staining of EpoR apparently localized within islets of chemosensitive cells of the carotid body was detected (Soliz et al., 2005). This observation implies that circulatory Epo interacts with carotid body cells, probably by binding the EpoR. In a next step, by using plethysmograph, we evaluated the hypoxic ventilatory response in WT animals previously injected with 2000 U/kg of rhEpo in the tail vein. We observed that Epo-injected mice showed higher respiratory frequency but lower tidal volume than saline-injected controls when exposed to hypoxia. Considering that a glycoprotein such Epo requires > 10 h in order to cross the blood–brain barrier (Statler et al., 2007), these results suggest that circulating Epo can activate peripheral chemoreceptors. More interesting, based on the fact that ovarian steroids can influence the expression of hypoxia-inducible genes, such as renal Epo, vascular endothelial growth factor, endotheline 1, nitric oxide synthase, and HIF-1 (Gassmann et al., 2009), we further hypothesized that gender-dependent regulation of hypoxic ventilation is mediated by erythropoietin. Indeed, our results in female previously injected with 2000 U/kg of rhEpo showed an increase in hypoxic ventilation, associated to increased respiratory frequency and tidal volume (Soliz et al., 2009). These results suggest that plasma Epo and sex female hormones interact in the carotid body cells under hypoxic conditions.

Considering that Epo is extensively used in clinics, similar experiments were performed in healthy human volunteers (27 ± 4 years). Acute intravenous injection of 5000 units of human recombinant Epo evoked an increase in the ventilatory response to hypoxia of women but decreased the ventilatory response to hypoxia in men subjects. While Epo is synthesized in testicular cells (Magnanti et al., 2001) and in turn testosterone modulates the hypercapnic and hypoxic ventilatory response (Emery et al., 1994; Tatsumi et al., 1994), it is tempting to suggest that Epo may have specific impact in the basal level of testosterone and/or in cells expressing testosterone receptors (Soliz et al., 2009).

5.2. Epo and carotid bodies during the postnatal development

Birth imposes a dramatic change in PO_2 supply in mammals, with arterial PO_2 normally rising to between 3-fold and 4-fold above in utero levels. Associated with this change in PO_2 availability is a well-documented resetting of PO_2 sensitivity of the carotid body. Although the carotid body exhibits little response to acute hypoxia at birth, PO_2 sensitivity gradually increases during the postnatal period (Carroll and Kim, 2005; Donnelly et al., 2005), with postnatal maturation occurring over the first 2 weeks in rats (Niane and Bairam, 2011,2012). This resetting matches the dynamic range of the carotid body to the higher postnatal PO_2 levels and contributes to a concomitant increase in the acute hypoxic ventilatory response of the animal, although central nervous system maturation is also critical to postnatal changes in the hypoxic ventilatory response (Bissonnette, 2000).

As mentioned above, mature erythropoietic response already exist at fetal life. Since the first fetal erythropoiesis occurs in liver (Palis et al., 2010), at the end of the gestational period, kidney is already able to produce Epo and the bone marrow produces hematopoietic stem cells able to differentiate and mature into definitive red cells (Palis et al., 2010). Indeed, increased hemoglobin and hematocrit levels are observed in postnatal rodents and humans exposed to acute or chronic hypoxia (Joseph et al., 2000). Moreover, as preterm infants experience anemia, secondary to postnatal suppression of hematopoiesis (similar to physiologic anemia in infancy), Epo is widely used at this age to normalize the levels of hemoglobin (Von Kohorn and Ehrenkranz, 2009).

Nevertheless, while the presence of these actors in the postnatal scene was established, still no work demonstrated that the circulating Epo modulates the carotid body-mediated ventilatory response to hypoxia. Lam and collaborators recently published the sole study concerning Epo in carotid bodies (Lam et al., 2009). By using immunohistochemistry technique this work shows that rat carotid body glomus cells secret Epo and express EpoR at the postnatal days 3, 14 and 28. Moreover, this work shows that postnatal intermittent and/or chronic hypoxia augment the expression of both Epo and its receptor (Lam et al., 2009). Obviously, this data support the hypothesis that circulating Epo modulates the ventilatory response to normoxia and hypoxia of carotid bodies during the postnatal development.

A number of compelling models including adenosine monophosphate-activated protein kinase, hemeoxygenasease-2, mitochondria and hydrogen sulphide (Lopez-Barneo et al., 2008), have been proposed as O_2 sensors in glomus cells. Whatever the molecular identity of the sensor itself, it is well known that once the reduction in arterial O_2 is detected, these cells rapidly depolarize (Lopez-Barneo et al., 2008). This depolarizing stimulus is of sufficient magnitude to cause an influx of Ca^{2+} through voltage-gated Ca^{2+} channels, which leads the release of neurotransmitters, including catecholamines (Lopez-Barneo et al., 2008). Accordingly, the study of oxygen-sensitive machinery in the carotid body was classically made by electrophysiological techniques. Similarly, the

molecular basis of Epo regulation of O_2 sensing by the carotid body can be investigated by using patch-clamp, to study the O_2-sensitive ion channels, Ca^{2+} imaging to study voltage-gated Ca^{2+} entry, and amperometry electrophysiological technique, to monitor the secretion of neurotransmitters by the oxidation of a carbon fibre. Finally, carotid bodies at postnatal ages are not overwrapped with connective tissue. Evidently this makes easer to find and work with them. As some of these experiments already initiated in my and others laboratories, new results will appear soon in the literature.

6. Conclusion and clinical remarks

The development of Epo to correct anemia in chronic kidney disease is a remarkable example of how basic science can lead to new paradigms for improving patient care and quality of life (Bradley and Denker, 2004). In recent years, it has however been demonstrated that Epo also plays a critical role in the development, maintenance, protection and repair of the nervous system (Kumral et al., 2011). Unlike ordinary blood transfusions (Kasat et al., 2011; Zuppa et al., 1995), subcutaneous treatment of preterm neonates with Epo offers the additional advantage of improving ventilatory function. This is in keeping with our previous work showing that cerebral Epo prevents hypoxia-induced respiratory depression in adult mice. Altogether, this evidence provides the basis for exploring the role of Epo in neuronal respiratory control during postnatal development. The immaturity of the respiratory control system remains one of the major causes of hospitalization and morbidity in pre-term babies and neonates. The recent data showing that erythropoietin in postnatal brain stimulates the central respiratory network upon hypoxia, combined with our results showing that Epo and its receptor are expressed in the carotid bodies of newborn rats are significant in that regard. Furthermore, this newly uncovered role of Epo might open new perspectives for the therapeutic use of Epo in the treatment of respiratory diseases characterized by limited O_2 availability (e.g. apnea of prematurity and respiratory distress syndrome), which are major causes of morbidity in both term and premature babies. In view of clinical trials in which high-dose recombinant human Epo is routinely and safely administrated (Jelkmann, 2005; Juul et al., 1999), we strongly believe that Epo will also be "rediscovered" as an effective treatment for several neonatal respiratory diseases.

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