Hypoxia

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REVIEW

Erythropoietin and the use of a transgenic model of erythropoietin-deficient mice

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Abstract: Despite its well-known role in red blood cell production, it is now accepted that erythropoietin (Epo) has other physiological functions. Epo and its receptors are expressed in many tissues, such as the brain and heart. The presence of Epo/Epo receptors in these organs suggests other roles than those usually assigned to this protein. Thus, the aim of this review is to describe the effects of Epo deficiency on adaptation to normoxic and hypoxic environments and to suggest a key role of Epo on main physiological adaptive functions. Our original model of Epo-deficient (Epo-TAg^{h}) mice allowed us to improve our knowledge of the possible role of Epo in O_2 homeostasis. The use of anemic transgenic mice revealed Epo as a crucial component of adaptation to hypoxia. Epo-TAg^{h} mice survive well in hypoxic conditions despite low hematocrit. Furthermore, Epo plays a key role in neural control of ventilatory acclimatization and response to hypoxia, in deformability of red blood cells, in cerebral and cardiac angiogenesis, and in neuro- and cardioprotection.

Keywords: Epo-TAg^{h} mice, mouse model, physiological functions, hypoxia

Introduction

Life is dependent upon transport and utilization of oxygen (O_2) for the metabolic conversion of nutrients into energy,[1] making O_2 homeostasis an essential process for survival. Indeed, an inadequate level of O_2 is detrimental for the tissues, and complex mechanisms serve to maintain in vivo the cellular O_2 concentration within a physiological range.[1] An imbalance between O_2 delivery and requirement, such as at high altitude, activates a variety of specific mechanisms at molecular, cellular, and systemic levels. High altitude is accompanied by low atmospheric O_2 pressure, which sequentially leads to insufficient O_2 uptake and reduced tissue oxygenation. Hypoxic exposure can be intermittent (obstructive sleep apnea) or continuous (high altitude, cardiorespiratory failure), leading to different strategies to address these stresses. Indeed, acute hypoxia triggers rapid and transient compensatory mechanisms, while chronic hypoxia (CHx) leads to more durable changes with gene expression modifications.[1] The hypoxia-inducible factor-1 (HIF-1) is the most important protein regulating homeostasis when O_2 is lacking.[2] Under hypoxia, stabilization of HIF-1 modulates the expression of hypoxia-regulated genes such as vascular endothelial growth factor (VEGF), glucose transporters, or erythropoietin (Epo).[1–5] Epo is a hematopoietic growth factor and represents the main regulator of erythropoiesis. In low O_2 conditions, the number of erythrocytes increases to sustain O_2 delivery.[6] This polycythemia, secondary to an increase in Epo release, is one of the key factors of acclimatization to CHx, through

the increase in O_2-carrying capacity.[7–9] However, despite this well-known role, it was suggested that Epo plays a key role in other physiological functions. Thus, Epo is also known to be fundamental for embryonic development,[10] and it is also largely involved in the normal functioning of most organs, including brain, heart, and muscles by regulating numerous cell functions such as calcium flux or cell survival.[11,12] To assess these pleiotropic actions of Epo on organs and cells, transgenic models of mice have been developed.[13,14]

The aim of this review is to provide a brief presentation of the physiological effects of Epo and describe the effects of Epo deficiency on adaptation to normoxic and hypoxic environments in an original model of Epo-deficient (Epo-TAg^h) mice. From these observations, we suggest a key role of Epo on multiple physiological adaptive functions, especially in response to hypoxia.

Epo and Epo-receptor expression in organs

Epo is a glycoprotein that is synthesized by peritubular fibroblasts in the kidneys of adults and in hepatocytes of fetal liver.[15] Epo was originally believed to play a role that restricted to stimulation of early erythroid precursor proliferation, inhibition of apoptosis, and differentiation of the erythroid lineage. Currently, in addition to its well-known role in red blood cell production, a diverse group of cells has been identified to produce Epo and/or Epo receptor (Epo-R) including endothelial cells, smooth muscle cells, retina tissues, testis, and cells of the central nervous system.[12,16–19] Moreover, Epo mRNA was also detected in lungs, testis, heart, and brain but not in skeletal muscles, intestine, or bone marrow of rodents.[20,21]

Epo and the brain

Epo mRNA is constitutively expressed in the mice brain,[22,23] and Epo-R mRNA and protein are also expressed in the brain of rodents.[23,24] Indeed, Epo and its receptors are found in the nervous system, including in neurons, astrocytes, and endothelial cells.[16,18,22,23,25–27] Epo/Epo-R couple is involved in neuroprotection,[28] promotes neural plasticity,[29] and could have a potentially antidepressor effect.[30,31] Furthermore, Epo/Epo-R pathway is required for normal brain development.[32] Indeed, Epo is required for neural progenitor cell proliferation,[33] and it avoids neural apoptosis by maintaining Bcl-2 and Bcl-xl expression.[34,35] Indirectly, Epo improves sensory, cognitive, and endocrine functions of the central nervous system through its erythropoiesis-stimulating effect, because it increases the O_2 supply to the brain. The direct effects of Epo are independent of erythropoiesis.[36] Epo has been demonstrated to have neuroprotective effects after ischemic, hypoxic, metabolic, neurotoxic, and excitotoxic stress in the nervous system. Epo operates at several levels within the central nervous system, including limiting the production of reactive O_2 species and glutamate, neurotransmission modulation, promotion of angiogenesis, prevention of apoptosis, reduction in inflammation, and recruitment of stem cells.[37–40] Moreover, Epo-R was localized in both brainstem respiratory centers and carotid bodies,[24] and it is involved in ventilatory regulations during hypoxic challenges.

Epo and the heart

As in the brain, animal models of ischemia and acute myocardial infarction have shown that Epo reduces infarct size and improves left ventricular (LV) function. These effects are mediated mostly through apoptosis inhibition by activating pro-survival pathways in the myocardium, mobilization of endothelial progenitor cells, and inhibition of migration of inflammatory cells as well as potent pro-angiogenic properties.[41] Moreover, recent studies reported that the heart could be a site of Epo production[42–44] and, in particular, that cardiac tissue reveals Epo gene and protein expression.[20,45,46] Epo-R expression is high in cardiac progenitor cells.[47] Epo has angiogenic and antiapoptotic effects in the heart,[48,49] and intraperitoneal injection of Epo promotes the differentiation of cardiac progenitor cells into endothelial cells.[45] Overall, Epo is known to have a cardioprotective effect through anti-apoptotic, anti-inflammatory, and angiogenic effects,[50] and it could act on the oxidative stress.[51] For example, Epo may enhance protein kinase B or Akt and protect the heart from ischemia–reperfusion injuries.[52] This effect of Epo on Akt could regulate cardiomyocyte mitochondrial biogenesis,[53] by acting on the Akt/endothelial nitric oxide (NO)-synthase pathway.[54] Recently, it has also been shown that acute Epo injection can efficiently improve resuscitation and survival rates in a model of cardiac arrest in pigs.[55,56]

Epo and the muscle

Epo-R has been identified in muscle biopsies suggesting a potential but undefined role of Epo on muscle metabolism or function.[57,58] In humans, these effects were suggested by excessive intake of Epo during endurance exercise in order to improve performance.[59] In some athletes, Epo increases performance during endurance exercises more than expected on the only basis of an increase in hematocrit. This suggests a potentially direct effect of Epo on muscle metabolism,[60] even if this effect remains to be validated.

Epo could induce angiogenesis and accumulation of VEGF in skeletal muscles submitted to ischemia–reperfusion experiments[61] and act as a promoter of growth factors in skeletal muscles.[57] In vitro, Epo-R mRNA and protein expression were identified in the primary muscle cells of mammals.[57,62,63] However, the effects of administration of human recombinant Epo (rhu-Epo) and the role of Epo-R in culture media remain controversial. Some authors showed a positive effect,[57] and some others showed no effect,[62,63] on primary myoblast proliferation. In vivo, although mRNA-encoding Epo-R and Epo-R proteins were detected in rats,[64] skeletal muscle treatment by rhu-Epo have shown controversial results. However, Epo is suspected to induce a shift in muscle fiber metabolism toward a more oxidative phenotype[65,66] and prevent the impairment of mitochondrial structure and function.[67] Moreover, some authors observed a positive effect of rhu-Epo on myoblast apoptosis,[64,68] proteolysis, glycolysis, and mitochondrial functions.[69] The authors suggested[70] that indirect effects of Epo treatment through the amelioration of O_2 supply could explain the observed effects of Epo on skeletal muscles during endurance exercises.

Epo underproduction and clinical disorders

In adults, insufficient Epo production results mostly from direct damage to Epo-producing cells in the kidneys or to a lesser extent from the suppression of Epo production by inflammatory cytokines.[71] Indeed, in patients suffering from rheumatoid arthritis, cancer, and acquired immune deficiency syndrome (AIDS), inflammatory cytokines suppress Epo gene expression.[71–75] In contrast, patients with renal failure generally develop anemia due to the suppression of erythropoiesis and to a moderate reduction in red cell life span.[71] Furthermore, the main reason of anemia in patients with uremia is an insufficient Epo production.[71,76,77] Diabetic nephropathy can also lead to Epo deficiency and anemia.[78] Moreover, exposure to metals such as cadmium or platinum results in a modification of the structure and function of renal proximal tube, resulting in the suppression of Epo production.[71] Patients with increased plasma viscosity due to monoclonal dysproteinemias have an inappropriate Epo production.[71,79]

Mouse model of Epo deficiency

A mouse model of Epo-TAg^h mice was used for the first time for the identification of the renal Epo-producing cells.[80] We then developed this model in 2006 in our laboratory to assess the potential roles of Epo and/or anemia in the adaptation processes to hypoxia.

The transgenic construct contains an SV40 sequence in the five untranslated region of the mouse Epo gene, which is flanked on each side by 9 and 7.5 kb of DNA from the mouse Epo locus.[80] Anemia-inducible Epo expression was observed in the kidneys.[80] Thus, these mice present a severe reduction in Epo gene expression,[13,80] leading to low plasmatic level of Epo (122±16 pg/mL in wild type [WT] vs 53±18 pg/mL in Epo-TAg^h mice) and thus chronic anemia with low hemoglobin concentration (17.1±0.3 g in WT vs 6.9±0.3 in Epo-TAg^h mice; Table 1). However, Epo-TAg^h mice have a good survival rate in CHx (14 days, 4,500 m) through an increase in ventilation and cardiac output.[81,82] They also develop cerebrovascular adaptations to chronic anemia and hypoxia.[83]

Characteristics of Epo-TAg^h mice in normoxic and hypoxic conditions: effect of Epo deficiency and/or anemia

Blood characteristics

Red blood cells are responsible for the transport of >98% of the O_2 into the blood. In order to deliver O_2 to the tissues, red blood cells deform as they enter capillaries. In Epo-TAg^h mice, hematocrit and hemoglobin are ~60% lower (Table 1) than those observed in WT mice.[82] Furthermore, Epo deficiency decreases blood viscosity and slightly reduces red blood cell deformability.[84] After CHx, hemoglobin concentration remained 60% lower in Epo-TAg^h mice as compared with that of WT mice,[81] despite a proportional increase in Epo concentration as compared with WT animals.[85] Nevertheless, in Epo-TAg^h mice, Epo concentration following acute hypoxia increased to reach only the normoxic WT value[83] and returned to basal value after CHx.[20]

Cardiac characteristics

Epo-TAg^h mice show right ventricular and LV hypertrophy,[82] leading to increased stroke volume and cardiac output (Table 1). This adaptive process, already described,[86] allows to offset the fall in arterial O_2 content due to anemia. Echocardiographic data confirmed compensatory LV hypertrophy, higher myocardial chamber volumes, and a higher cardiac output,[20] which could be explained by an increase in preload without change in left ventricle afterload as depicted by unchanged blood pressure. Although cardiac output was increased in Epo-TAg^h mice, O_2 delivery remained lower than in control WT animals. Furthermore, Epo/Epo-R pathway is known to be involved in the transcription of target genes that mainly involved in the inhibition of apoptosis and cell

Table 1 Characteristics of wild-type and Epo-TAg^h male mice in normoxic conditions

		References	Wild-type mice	Epo-TAg^h mice
	Hemoglobin ($g \cdot dL^{-1}$)[b]	Macarlupu et al[81]	17.1±0.3	6.9±0.3[a]
	Hematocrit (%)[b]	Macarlupu et al[81]	54.2±0.8	24.0±1.6[a]
Cardiac characteristics	Heart rate (bpm)[c]	El Hasnaoui-Saadani et al[20]	500±27	548±46
	Stroke volume ($\mu L \cdot g^{-1}$)[c]	El Hasnaoui-Saadani et al[20]	1.9±0.19	3.2±0.68[a]
	Cardiac output ($mL \cdot min^{-1} \cdot g^{-1}$)[c]	El Hasnaoui-Saadani et al[20]	0.94±0.14	1.76±0.43[a]
	Systolic blood pressure (mmHg)[c]	El Hasnaoui-Saadani et al[20]	96.7±8.5	94.2±5.8
	Right ventricular weight (mg)[b]	Macarlupu et al[82]	23±1	33±2[a]
	Left ventricular and septum weight (mg)[b]	Macarlupu et al[82]	80±2	113±3[a]
	Fulton ratio[b]	Macarlupu et al[82]	0.288±0.013	0.297±0.010[a]
Ventilatory parameters	Minute ventilation ($mL \cdot min^{-1} \cdot g^{-1}$)[c]	Voituron et al[90]	2.26±0.48	2.17±0.53
	Respiratory frequency ($c \cdot min^{-1}$)[c]	Voituron et al[90]	261±34	284±54
	Tidal volume ($\mu L \cdot g^{-1}$)[c]	Voituron et al[90]	8.63±1.26	7.62±1.09
	Resting oxygen consumption ($mL \cdot min^{-1} \cdot kg^{-1}$)[b]	Macarlupu et al[81]	93.3±4.7	96.8±6.5
	Maximal oxygen consumption ($mL \cdot min^{-1} \cdot kg^{-1}$)[b]	Macarlupu et al[81]	270.7±22.0	210.2±12.3[a]

Note: [a]Indicates significant difference between wild-type and Epo-TAg^h mice. [b]Data presented as mean ± SEM. [c]Data presented as mean ± SD.
Abbreviations: Epo, erythropoietin; Epo-TAg^h mice, Epo-deficient mice; SD, standard deviation; SEM, standard error of the mean.

proliferation[87] through the phosphorylation of Jak2 and STAT-5. Because of unchanged P-STAT-5/STAT-5 ratio, we could not confirm the activation of this cardioprotective pathway in response to chronic Epo deficiency.[20] In addition to its angiogenic function, VEGF may also activate pathways associated with NO synthesis and thus induce vasodilation, improving blood supply to cardiac cells.[88] Thus, we demonstrated that chronic Epo deficiency induces a cardiac angiogenesis probably mediated by HIF-1α/VEGF (Figure 1) and Epo-R pathways, which could optimize O_2 supply and limit the consequences of chronic anemia on cardiac cells.

The effects of both chronic Epo deficiency and hypoxia on myocardial contractile function and cardioprotective processes were investigated using Epo-TAg^h mice.[20] The initiation of cardioprotective mechanisms was estimated through Epo-R/P-STAT5 signalization as well as by a change in the P-STAT5/STAT5 ratio in response to chronic Epo deficiency and/or hypoxia. When 14 days of CHx were added to Epo deficiency, the expected cardiac hypertrophy[89] was reduced and cardiac output could not catch up with the O_2 demand. Systolic blood pressure did not increase indicating that systemic afterload was not responsible for the decrease in cardiac output. Moreover, CHx did not significantly affect right ventricular hemodynamics, and Epo-TAg^h mice did not develop pulmonary hypertension. Therefore, the decrease in cardiac output was not the consequence of right ventricular failure. After CHx, Epo-TAg^h mice displayed a lower LV hypertrophy than the normoxic anemic mice, which could account for the decrease in cardiac output and O_2 delivery. Furthermore, our data showed a mild alteration of diastolic and systolic LV function. These results suggested that altered myocardial function in Epo-TAg^h mice exposed to CHx could participate in the failure of cardiac adaptation in this severe condition.

Although hypoxia or Epo-deficiency leads to an overexpression of HIF-1α, VEGF (Figure 1), Epo, and Epo-R, we did not observe a synergic effect of these combined constraints on the heart of Epo-TAg^h mice exposed to CHx, except for P-STAT-5/STAT-5 ratio. However, this ratio was lower in Epo-TAg^h than in WT mice exposed to CHx, suggesting that the activation of the cardioprotective pathways downstream the Epo/Epo-R system may represent a limiting step. As we found a decrease in LV hypertrophy and functional LV adaptation, a depressed HIF-1α/VEGF pathway (Figure 1) as well as a reduced O_2 delivery, we suggested that cardiac adaptive mechanisms that take place with chronic Epo deficiency and hypoxia might require extensive Epo effects (angiogenesis, cardioprotection) on the heart.

Ventilatory and metabolic characteristics

Our first publication on the Epo-TAg^h mice model showed a greater ventilation in normoxia in these anemic mice as compared to WT animals.[82] This difference was mainly due to a larger tidal volume. However, more recently, we have not observed a baseline ventilatory adaptation in Epo-TAg^h male mice in normoxia (Table 1, Figure 2) when compared with that of WT mice.[90] Some explanations could be proposed to explain this difference. First, in the study of Macarlupu et al, the control animals were classical WT animals and not littermates, and it is well known that the environmental conditions during the first day of life could induce significant changes in ventilatory variables.[91] Second, the method used to measure ventilatory variables can induce slight differences, according to the plethysmograph used, for example. Third,

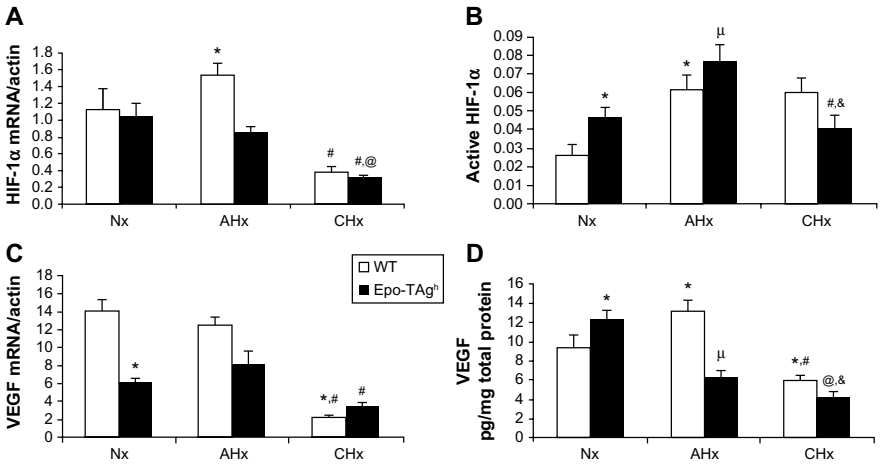

Figure 1 Cardiac angiogenesis in Epo-TAg^h mice.
Notes: HIF-1α and VEGF mRNA (**A**, **C**) and protein (**B**, **D**) expression in the heart of WT (white bar) and Epo-TAg^h (black bar) mice in normoxia and after 48 hours (AHx) or 14 days (CHx) of hypoxic exposure. Epo deficiency led to a rise of angiogenesis through the HIF-1α and VEGF pathway activation. Epo-TAg^h mice were not able to maintain cardiac adaptation to hypoxia during the long-term exposure. Values are expressed as mean ± SEM. *P<0.05 vs Nx WT; &P<0.05 vs Nx Epo-TAg^h; #P<0.05 CHx vs AHx; @P<0.05 CHx Epo-TAg^h vs CHx WT. µP<0.05 AHx Epo-TAg^h vs AHx WT. Reprinted from *Respir Physiol Neurobiol*, Volume 186(2), El Hasnaoui-Saadani R, Marchant D, Pichon A, et al, Epo deficiency alters cardiac adaptation to chronic hypoxia, pages 146–154. Copyright 2013 with permission from Elsevier.[20]
Abbreviations: AHx, acute hypoxia; CHx, chronic hypoxia; Epo, erythropoietin; Epo-TAg^h mice, Epo-deficient mice; HIF-1α, hypoxia-inducible factor-1α; Nx, normoxia; SEM, standard error of the mean; VEGF, vascular endothelial growth factor; WT, wild type.

it could be possible that Epo-TAg^h mice could have exhibited some epigenetic adjustments between 2006 and 2014. Indeed, more than 30 generations of mice have lived between the first and the last experiment, and it is not impossible that epigenetic changes would improve the whole O_2 transport steps, and thus, ventilatory adaptation could appear in the last generations of mice.[92]

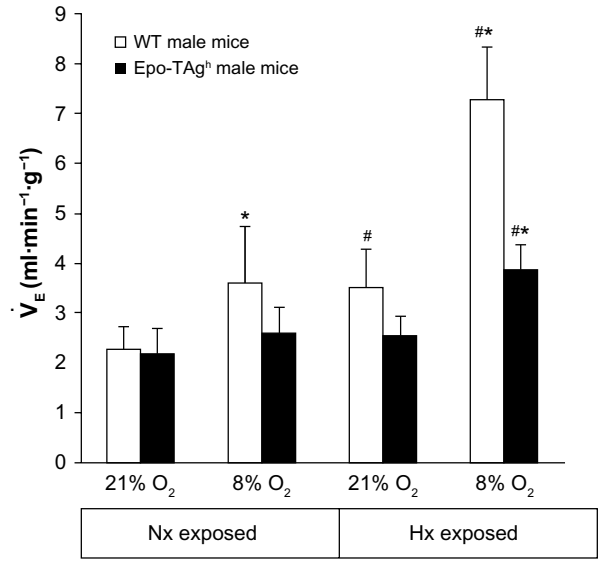

Figure 2 Ventilatory response to hypoxia in Epo-TAg^h mice.
Notes: Minute ventilation measured in normoxia (F_IO_2 21%) or acute hypoxia (F_IO_2 8%) in WT (white bar) and Epo-TAg^h (black bar) mice maintained in normoxic (Nx exposed) or hypoxic (14 days, Hx exposed) conditions. Epo-TAg^h mice had a normal ventilation at rest, did not display ventilatory acclimatization to hypoxia, and did not respond to acute hypoxia even after the exposure to chronic hypoxia. Values are expressed as mean ± SD. *P<0.05 21% O_2 vs 8% O_2; #P<0.05 Nx exposed vs Hx exposed, same strain, same F_IO_2. Adapted from Voituron N, Jeton F, Cholley Y, et al. Catalyzing role of erythropoietin on the nitric oxide central pathway during the ventilatory responses to hypoxia. *Physiol Rep*. 2014;2(2):e00223. © 2014 Voituron N, Jeton F, Cholley Y, et al. *Physiological Reports* published by Wiley Periodicals, Inc. on behalf of the American Physiological Society and The Physiological Society.[90]
Abbreviations: Epo, erythropoietin; Epo-TAg^h mice, Epo-deficient mice; Hx, hypoxia; Nx, normoxia; SD, standard deviation; WT, wild type.

In female Epo-TAg^h mice, we observed a difference in respiratory frequency and minute ventilation with larger values for the Epo-TAg^h mice as compared with those of WT mice (unpublished data). There is no change in resting O_2 consumption ($\dot{V}O_2$) in Epo-TAg^h mice, while $\dot{V}O_2$ max is only 30% reduced (Table 1) as compared with that of WT mice,[82] despite a 60% reduction in hemoglobin concentration. The normal resting $\dot{V}O_2$ in anemic Epo-TAg^h mice could also be explained by the elevated cardiac output associated with better tissue extraction of O_2,[86] which could compensate for the decrease in O_2 transport capacity. Severe anemia also generally induces a reduction in physical performance.[93–95] In Epo-TAg^h mice, the reduction in $\dot{V}O_{2max}$ was only moderate, suggesting compensatory mechanisms such as an increase in maximal cardiac output, augmented capillarization, and better O_2 extraction.

The normal ventilatory response to acute hypoxia is characterized in adult mammals by a hyperventilation followed by a relative ventilatory decline named "roll off".[96,97] If hypoxia persists, an increase in ventilation occurs (ventilatory acclimatization to CHx),[98] which is accompanied by an increase in the sensitivity of the respiratory control system.[99–101] Epo-TAg^h mice displayed neither ventilatory response to acute hypoxia nor ventilatory acclimatization to CHx (Figure 2).[90] However, after 14 days of exposure to chronic hypoxia, Epo-TAg^h mice increased their ventilation when exposed acutely to a hypoxic stress (Figure 2;

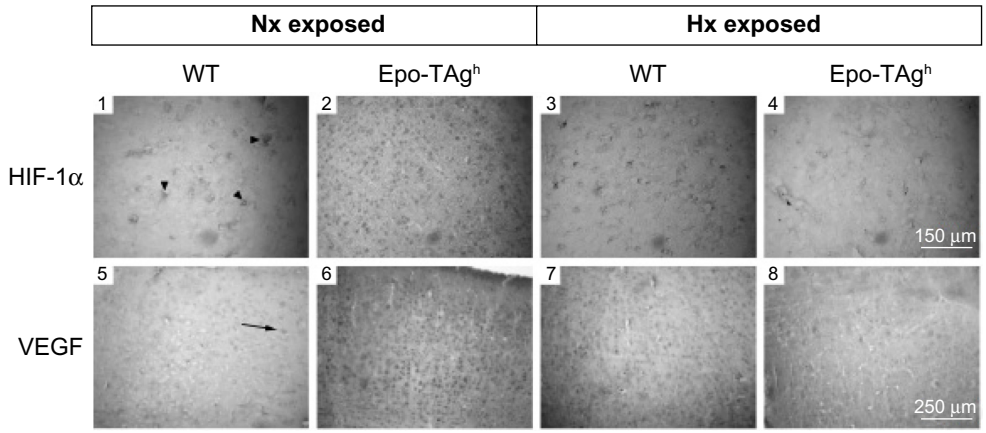

Figure 3 Cerebral angiogenesis in Epo-TAg^h mice.
Notes: Immunohistological detection of HIF-1α and VEGF at the sensory cortex level in normoxia (Nx exposed) and after chronic hypoxia exposure (Hx exposed) in WT (**1, 3, 5, 7**) and Epo-TAgh mice (**2, 4, 6, 8**). Arrowheads and arrow indicate HIF-1α (1)- and VEGF (**5**)-positive cells, respectively. In normoxia, Epo-TAgh mice showed an increase in HIF-1α (2)- and VEGF (**6**)-positive cells suggesting an enhancement of cerebral angiogenesis through the HIF-1α /VEGF pathway. In WT mice, chronic hypoxia led to an increase in HIF-1α (**3**) and VEGF (**7**), while they led a decrease in Epo-TAgh mice (**4, 8**). Adapted from *Am J Physiol Regul Integr Comp Physiol*. Volume 296(3). El Hasnaoui-Saadani R, Pichon A, Marchant D, et al. Cerebral adaptations to chronic anemia in a model of erythropoietin-deficient mice exposed to hypoxia. Pages: R801–R811. Copyright 2009.[83]
Abbreviations: Epo, erythropoietin; Epo-TAg^h mice, Epo-deficient mice; HIF-1α, hypoxia-inducible factor-1α; Hx, hypoxia; Nx, normoxia; VEGF, vascular endothelial growth factor; WT, wild type.

8% O_2, 5 minutes).[90] These results differ from those previously published.[82] We cannot exclude the fact that our transgenic mice, along generations, developed adaptation strategies to cope with Epo deficiency and/or chronic anemia.

Brain adaptations

Epo deficiency in Epo-TAg^h mice leads to cerebral adaptations (Figures 3 and 4).[83] Indeed, in the brain of these normoxic mice, we observed an increase in the transcript and the protein levels of HIF-1α, VEGF (Figure 3), Epo-R (Figure 4), and P-STAT-5/STAT-5 ratio accompanied with an increase in cerebral capillary density. Taken together, these data suggest that Epo-TAg^h mice have developed cerebral angiogenesis, probably via the HIF-1α/VEGF pathway (Figure 3), optimizing O_2 diffusion as previously described.[83,102] Furthermore, the increase in P-STAT-5/STAT-5 ratio in the brain suggests neuroprotective mechanisms and angiogenesis with a decrease in apoptosis and an increase in cell proliferation.[23,103] Overall, these results illustrate the direct and indirect effects of Epo in terms of O_2 delivery improvement and the activation of neuroprotective mechanism to counteract the lack of Epo in the brain.

Skeletal muscles

Our model of transgenic Epo-TAg^h mice has allowed us to study the role of Epo on skeletal muscle development, angiogenesis, and acclimatization to hypoxia. Our main

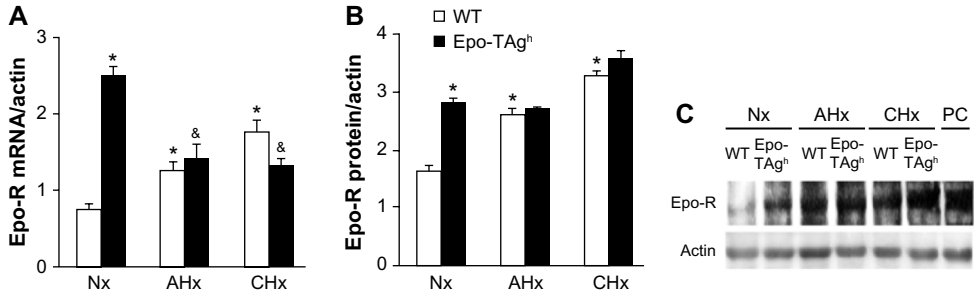

Figure 4 Effect of Epo deficiency on Epo-R expression in cerebral cortex.
Notes: Quantitative determination of Epo-R in the cerebral cortex of WT and Epo-TAg^h mice in Nx and following AHx and CHx. Epo-R mRNA (**A**) and protein level (**B**) are shown next to their corresponding protein bar graphs. Representative Western blot of Epo-receptor (Epo-R) (**C**). Values are expressed as mean ± SD. *P<0.05 vs Nx WT; &P<0.05 vs Nx Epo-TAgh. Adapted from *Am J Physiol Regul Integr Comp Physiol*. Volume 296(3). El Hasnaoui-Saadani R, Pichon A, Marchant D, et al. Cerebral adaptations to chronic anemia in a model of erythropoietin-deficient mice exposed to hypoxia. Pages: R801–R811. Copyright 2009.[83]
Abbreviations: AHx, acute hypoxia; CHx, chronic hypoxia; Epo, erythropoietin; Epo-R, Epo receptor; Epo-TAg^h mice, Epo-deficient mice; Nx, normoxia; SD, standard deviation; WT, wild type; PC, peptide control.

Table 2 Main characteristics of Epo-TAg^h mice when compared with those of wild-type mice

	Blood	Heart	Ventilation	Brain	Muscle
Normoxia	Low Epo, low Hb	High CO, high angiogenesis		High angiogenesis	High microvessel network
Acute hypoxia			Low HVR		
Chronic hypoxia	Low Hb	Low cardioprotection	Low VAH		High microvessel network

Abbreviations: CO, cardiac output; Epo, erythropoietin; Epo-TAg^h mice, Epo-deficient mice; Hb, hemoglobin; HVR, hypoxic ventilatory response to hypoxia; VAH, ventilatory acclimatization to hypoxia.

results displayed a developmental adaptation to Epo deficiency and/or chronic anemia by an improvement of microvessel network (Table 2) in both fast and slow skeletal muscles.[85] This adaptation of Epo-TAg^h mice was not accompanied by any difference in skeletal muscles for contractile structure, metabolism, maximal strength, fatigability, contraction time, and relaxation time. Moreover, we never observed any overexpression of HIF-1α or VEGF protein. The discrepancy between our results and those of Mille-Hamard et al's team[69] could be explained by a genetic developmental adaptation to chronic anemia in our transgenic model. In the Mille-Hamard et al's transgenic model, Epo deficiency is induced by vaccination of adult mice, so that developmental adaptation has not occurred. When exposed to severe chronic hypobaric hypoxia (4,300 m) during 14 days, skeletal muscles of Epo-TAg^h mice were not submitted to deconditioning. Neither skeletal muscle phenotype nor skeletal muscle functions were altered compared with those of WT mice.[104] In the skeletal muscles of our Epo-TAg^h model, we did not detect Epo mRNA in both normoxia and hypoxia. Moreover, the hypoxia-induced elevation of circulating Epo was not correlated with an increase in Epo concentration in skeletal muscle. Overall, these results favor the hypothesis of an indirect effect of Epo on skeletal muscles. We clearly show that, in our model, the deficit in oxygenation caused by anemia is responsible for the main change in skeletal muscles such as the improvement of microvessel network (Table 3). Unfortunately, we cannot study the regeneration process of the Epo-TAg^h model, because in this mutant, Epo has been replaced by antigen T, which has been shown to alter skeletal muscle regeneration.[105]

Limits of our models

Experiments were performed on whole-body Epo-TAg^h mice[13] that display a very low hematocrit (20%) and did not develop polycythemia after CHx.[81] Therefore, it is important to note that our model of transgenic mice combines the effects of chronic anemia (low O_2 content) and the effects of chronic Epo deficiency. To differentiate the respective effect of each constraint is rather difficult since chronic anemia itself is a consequence of chronic Epo deficiency. However, it could be speculated from the activation of HIF/VEGF systems that the reduction in tissue O_2 delivery (and therefore chronic anemia) is the main trigger of the observed adaptations.

Conclusion

For many years, Epo was mainly considered a growth factor for erythropoiesis and a determinant factor for the acclimatization to CHx only through an increase in O_2 transport capacity. It appears from recent studies that it may also participate in the acute and chronic responses to hypoxia (Table 2) through the activation of Epo-Rs in various organs (brain, heart, muscle, chemoreceptors). However, there is still a lot of debates and uncertainties about the presence and functionalities of these receptors. Our model of Epo-TAg^h mice may help to unravel a possible key role of Epo in O_2 homeostasis. Indeed, our studies demonstrate that high levels of Epo are not necessary for survival in chronic moderate hypoxia. Moreover, we showed that Epo could play a key-regulating role in the neural control of ventilatory acclimatization to hypoxia and hypoxic ventilatory response probably via a catalyzing role on the NO central pathway. We also demonstrated that chronic Epo deficiency induced cerebral and cardiac angiogenesis, which could have synergic effects not only in neuro- and cardioprotection but

Table 3 Indices of the microvascular supply of the deep region of gastrocnemius muscle fibers in normoxia and after chronic hypoxia

	Normoxia		Chronic hypoxia	
	WT mice	Epo-TAg^h mice	WT mice	Epo-TAg^h mice
Capillary density (cap/mm^2)	1699±350	1587±180	1845±393	1701±211
Capillary-to-fiber ratio	2.23±0.29	2.69±0.29[a]	2.28±0.20	2.62±0.29[a]
Number of capillaries around single fibers	5.20±0.78	6.27±0.54[a]	5.22±0.82	6.19±0.41[a]
Individual capillary-to-fiber ratio	2.16±0.26	2.46±0.35[a]	2.15±0.19	2.58±0.21[a]

Notes: [a]Indicates significant difference between WT and Epo-TAgh mice. Data presented as mean ± SD.
Abbreviations: Epo, erythropoietin; Epo-TAg^h mice, Epo-deficient mice; WT, wild type.

also in O_2 supply optimization, in order to limit the consequences of chronic anemia on cerebral and cardiac tissues. However, under both constraints (chronic Epo deficiency and hypoxia), angiogenesis, neuroprotective, and cardioprotective pathways along with a functional LV adaptation failed to occur, showing the limits of these adaptive processes in heart and brain, but more importantly suggesting a crucial role of Epo in main physiological functions.

Some future areas of research could focus on the role of Epo as a global regulator of the cardioventilatory adaptations from erythrocytes synthesis, blood hemorheology, blood volume regulation, and ventilatory control.[106] Epo seems to be able to protect tissues (cardiomyocytes, lung, neurons) from various aggressions such as hypoxia, ischemia–reperfusion, or inflammation.[107] Moreover, there are few data on the possible effect of Epo on the ventilatory response to hypercapnia in contrary to the response to hypoxia. The role of Epo/Epo-R on the oxidative stress needs also to be studied later as this is central on tissue antiapoptosis properties.

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Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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