Chapter 9

Adaptations to Chronic Hypoxia Combined with Erythropoietin Deficiency in Cerebral and Cardiac Tissues

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Abstract

Chronic anemia-induced hypoxia triggers regulatory pathways that mediate long-term adaptive cardiac and cerebral changes, particularly at the transcriptional level. These adaptative mechanisms include a regulated cerebral blood flow and cardiac output, angiogenesis and cytoprotection triggered by hypoxia-inducible factor 1 alpha (HIF-1α), vascular endothelial growth factor (VEGF), neuronal nitric oxide synthase (nNOS) and Epo pathways. All these compensatory mechanisms aim to optimize oxygen delivery and to protect the brain and heart from hypoxic injury. We reviewed the effects of chronic hypobaric hypoxia as well as chronic anemia in the heart and brain, and we compared for the first time the effects of chronic hypobaric hypoxia combined with a severe lack of Epo (chronic anemia) in these vital organs. Functional cardiac adaptations such as cardiac hypertrophy, increased cardiac output as well as angiogenesis occurred along with the activation of HIF1α/VEGF and Epo/EpoR pathways under chronic anemia or hypoxia. Similarly, cerebrovascular adaptations take place through the same molecular mechanisms under chronic hypoxia or anemia. However, when both arterial pressure and content of oxygen are decreased, the cerebral and cardiac adaptative mechanisms showed their limitations. In addition, cerebral and cardiac cell injuries may have occurred following the combined effect of chronic anemia and hypoxia. By emphasizing the anemia and hypoxia-induced cerebral and myocardial adaptations, this review highlighted the crucial role of Epo in its non-erythropoietic functions such as angiogenesis and neuroprotection. Indeed, a better understanding of these protective mechanisms is of great clinical importance to the development of new therapeutic strategies for the management of ischemic heart and brain.

Keywords: chronic hypoxia, chronic anemia, angiogenesis, cardiac function, cerebral blood flow, oxygen homeostasis, neuroprotection, HIF-1-VEGF-Epo

1. Introduction

Inadequate level of oxygen like in chronic hypoxia or anemia is especially detrimental for cerebral and heart tissues. Indeed, hypoxia plays an important role in the pathogenesis of cerebral and myocardial ischemia, and chronic heart and lung diseases [1]. That is why specific mechanisms at a systemic, cellular and molecular level take place to maintain the oxygen homeostasis. It is important to clearly distinguish the differences between hypoxia and anemia. Hypoxia is a reduction of arterial pressure of oxygen (PaO_2), while anemia is a reduction of arterial content of oxygen (CaO_2) as it occurs during a decrease in hemoglobin concentration. This review focus mainly on chronic hypobaric hypoxia that occurs at high altitude and chronic anemia is referred to our model of transgenic mice that presents a constitutive erythropoietin deficiency. Furthermore, emphasis is placed on effects of chronic hypoxia and anemia in the cerebral and cardiac tissues. The discussion is mostly based on animal studies unless otherwise indicated, even though similarities of adaptative mechanisms are shown by human studies [2, 3].

Chronic hypoxia promotes angiogenesis by modulating the transcriptional regulator hypoxia-inducible factor 1 alpha (HIF-1α), which in turn triggers the upregulation of the erythropoietin [4], a major factor of acclimatization to hypoxia. HIF-1α is a master regulator of the hypoxic response and its proangiogenic activities include regulation of vascular endothelial growth factor (VEGF), but also Epo and its receptors (EpoR) [1, 5]. Erythropoietin primarily regulates red blood cell formation, and Epo serum levels are increased under hypoxic stress (e.g., anemia and altitude) [6]. However, several non-hematopoietic functions of Epo and its receptors have been exposed by experimental studies using genetically modified mice [7]. It is of great clinical importance that Epo has been shown to have protective functions in many different tissues. Indeed, these studies using recombinant human EPO (rHuEPO) suggested new therapeutic indications of Epo for the management of ischemic injuries of several tissues such as myocardium and brain [8–10].

Epo-induced angiogenesis may lead to an improvement in brain perfusion since Epo protects vascular bed integrity and stimulates angiogenesis [11–14] by acting indirectly on endothelial cells via activation of VEGF/VEGF receptor system, which is the most important regulator of endothelial growth and angiogenesis. Furthermore, Epo may have a positive effect on cerebral vasculature in addition to the cerebral blood flow (CBF) through alteration in nitric oxide (NO) production, which mainly derived from arginine and catalyzed by endothelial nitric oxide synthase (eNOS) [15]. Therefore, it seems that cellular protection and angiogenesis in heart and brain tissues are the dual role of erythropoietin and VEGF. These both cytokines are triggered by HIF-1α to maintain an adequate cellular oxygen supply and protect the brain and heart against hypoxic and or anemic injuries.

Our model of erythropoietin-SV40 T antigen (Epo-TAg^h) transgenic mice has a targeted disruption in the 5′ untranslated region of the Epo gene that dramatically reduces its expression. The homozygous animals are thus severely and chronically anemic [16, 17]. Therefore, these transgenic anemic mice provided us an interesting model to study the adaptive mechanisms to chronic anemia and hypoxia, especially in vital organs, such as brain and heart. The present

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review aims to give a brief synthesis of adaptations to chronic hypoxia in the brain and heart tissues, in absence of Epo. The first part will briefly describe the similarities of the signaling process of hypoxia-induced angiogenesis, as well as the other mechanisms that take place to protect the brain and heart against anemic and hypoxic injuries. The second part will focus on these adaptations in response to chronic anemia due to Epo deficiency (in comparison with adaptations induced by hypoxia). The third part will mainly describe the effect of both constraints (anemia and hypoxia) in cerebral and cardiac tissues.

2. Adaptations to chronic hypoxia in cerebral and cardiac tissues

2.1. Brain under chronic hypoxia

In the central nervous system, cerebrovascular and energy metabolism adaptations occur under hypoxic conditions in order to preserve an adequate tissue oxygen supply needed to support an optimal neuronal function. An acute hypoxic exposure triggers both a CBF and glucose consumption increases [18]. The stabilization of HIF-1α rapidly up regulates the vasodilatory enzyme inducible nitric oxide synthase (iNOS) [19]. NO, the enzymatic product of iNOS, relaxes vascular smooth muscle cells, providing a short-term increase in blood flow. Thus, an increase in cerebral NO following a rise in NOS isoforms expression is most probably responsible for the rise in CBF [20–23]. With longer hypoxic exposure, polycythemia and cerebral angiogenesis take place to enhance cerebral oxygenation, while cerebral NO level returns to basal value [23, 24]. Indeed, in a chronic hypoxic challenge to the central nervous system, the cerebral cortex is known to undergo a significant cerebrovascular remodeling, in order to preserve tissue oxygen and energy supply [24–29]. These microvascular changes occur relatively late compared to the physiological adaptations [30]. In the rat brain, the capillary density almost doubles, and the average intercapillary distance decreases from about 50 to about 40 μm [31]. Also, by 3 weeks of adaptation, the initial hypoxic-induced increased flow returns to baseline by 5 days [22], concomitantly there is an increasing hematocrit, glucose consumption is slightly elevated by about 15% [32–34] and tissue oxygen tension is restored [35, 36]. Finally, it is well accepted that blood flow alterations serve acute changes in oxygen delivery, while persistent changes are due to capillary density adjustments.

Molecular mechanisms underlying hypoxia-induced capillary increases are now well documented and involve specifically HIF-1α/VEGF pathway [23, 26, 33, 37, 38]. The HIF pathway regulates a host of pro-angiogenic genes, including VEGF, angiopoietin-1, angiopoietin-2 (Ang-2) and many others [39, 40]. HIF-regulated pro-angiogenic factors execute the HIF-specific angiogenic program by increasing vascular permeability (most probably through interaction with NO [41]), endothelial cell proliferation, sprouting, migration, adhesion and tube formation. In rats, HIF-1α is detected in the brain, in all cell types, shortly after the onset of hypoxia and persists for at least 2 weeks, until cerebral angiogenesis is completed within 3 weeks of exposure to hypoxia [23, 29, 35]. Brain angiogenesis also requires additional pro-angiogenic factors such as Ang-2. Ang-2, which is not constitutively expressed under normoxic conditions, is upregulated in rat and mouse endothelial cells following hypoxia [28, 38]. Ang-2 induction

during hypoxia is known to occur independently of HIF-1 and is due to cyclooxygenase-2 (COX-2) enzyme activity [42, 43]. More recent results also demonstrated that the hypoxic capillary response in aged mice was preserved after 3 weeks of hypoxia despite a significant delay in the response during the first week of exposure to hypoxia [25].

2.2. Heart under chronic hypoxia

In humans, the most characteristic and important cardiovascular response to hypoxia is pulmonary vasoconstriction, which reduces the caliber of pulmonary vessels and raises vascular resistance in a region of low alveolar PO_2. However, severe hypoxia has a direct deleterious effect on cardiac function. Hypoxic pulmonary vasoconstriction can cause chronic pulmonary hypertension. Myocardial contractility and maximum output are diminished during conditions of reduced oxygen supply. While maximum oxygen consumption is reduced in chronic hypoxia, cardiac output (CO) remains normal at rest, owing primarily to an increased red blood cell mass [44, 45].

In our animal model, we showed that all parameters of cardiac function were preserved when comparing wild-type (WT) mice under normoxic and chronic hypoxic conditions. Indeed, systolic blood pressure was not affected by 14-day hypoxia at 4500 m, and hypoxic wild-type mice did not develop pulmonary hypertension. Moreover, there was no cardiac hypertrophy at variance with what was shown in rats or humans in similar hypoxic conditions [46]. Moreover, cardiac output was not affected by chronic hypoxia alone and oxygen delivery was maintained. In addition, hypoxic wild-type mice responded by increasing plasma Epo and blood hemoglobin, resulting in a rise in oxygen-carrying capacity [47].

Furthermore, many reports now stated that heart could be an additional Epo productive tissue [48, 49]. Hoch et al. first showed an Epo gene and protein expression in cardiac progenitor cells [50]. Through specific binding to its receptor EpoR, Epo triggers intracellular signaling events that depend on the activation of Jak2 tyrosine kinase [51]. The exploration of these pathways revealed that Epo is also an angiogenic as well as an anti-apoptotic factor as described respectively in the brain [52] and the heart [47, 50, 53, 54]. As previously described [55], chronic hypoxia led to the activation of HIF-1α/VEGF pathway in the heart of adult wild-type mice most probably responsible for their enhanced myocardial angiogenesis. Also we demonstrated the activation of cardioprotective pathways, involving HIF-1α and Epo, as suggested by the increase of EpoR expression and P-STAT-5/STAT-5 ratio [47]. Furthermore, we could not exclude a cardiac metabolic gene remodeling since temporal changes in glucose metabolic genes in response to moderate hypobaric hypoxia [56] have been demonstrated.

However, these adaptive responses contribute to maintain an adequate tissue oxygen supply for the preservation of cardiac function and to protect the heart against hypoxic insults.

3. Adaptations to chronic Epo deficiency in cerebral and cardiac tissues

Anemia is defined as a lack of oxygen-carrying red blood cells which also results in a lack of oxygen delivery to tissue. The physiological and molecular responses to tissue hypoxia are

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increasingly understood while the effects of anemia are still poorly documented [57]. Our model of erythropoietin-SV40 T antigen (Epo-TAg^h) transgenic mice presents a severe reduction of Epo expression that induces chronic anemia [16, 17]. The first studies demonstrated that Epo-Tag^h mice could survive in chronic hypoxia (14 days at 4500 m), in part through an increase in ventilation and probably a higher cardiac output as suggested by a significant cardiac hypertrophy [58–60]. Hence, it was of interest of our team to compare the physiological and cellular responses to chronic anemia (low Hb) and chronic hypoxia (low P_aO_2) in cerebral and cardiac tissues. Indeed, the objectives of our studies were to determine if chronic anemic mice developed compensatory mechanisms in the brain and heart (vascular remodeling, adaptative function, pathways involving HIF-1α) to offset the decrease in hemoglobin concentration.

3.1. Brain under chronic anemia

Low O_2 environment is the principal regulator of HIF activity. The HIF pathway mediates the primary cellular responses to low O_2, which promotes both short- and long-term adaptation to hypoxia as already described in the previous paragraph. In this regard, we considered that anemia-induced cerebral hypoxia involved the same hypoxic molecules. It has already been described that anemia increases cerebral hypoxic genes expression such as HIF, VEGF and nNOS which are involved in O_2 homeostasis [61]. Indeed, studies on severe hemodilution using NOS-deficient mice showed an increased expression of HIF and nNOS proteins in the brain as well as an increased whole body HIF activity [57, 61–64]. Many other molecules, including Epo, VEGF and iNOS have also been shown to be upregulated in the anemic brain [61, 65]. Thus, it seems that during anemia, HIF-1α has the potential to regulate cerebral cellular responses under both hypoxic and normoxic conditions [23, 47, 57, 64, 65].

Then, we focused on potential mediators of the increase in CBF associated with both hypoxia and anemia. Indeed, local production of NO by endothelial NOS (eNOS), and nNOS mediates CBF under a number of physiological conditions, including anemia and hypoxia [24, 61, 63, 64]. Relatively specific inhibition of nNOS has been demonstrated to impair the increase in CBF associated with acute anemia [61] and hypoxia [66–68], implicating nNOS as an important mediator of CBF in both cases [63, 64]. In our studies, we also found an increase in nNOS, while iNOS and eNOS were unchanged but no corresponding change in cerebral NO concentration. The stabilization of HIF-1α, as already described in the brain in acute [61, 69] and chronic anemia [23], promote VEGF-induced angiogenesis as shown in normoxic Epo-TAg^h mice with a rise in the capillary/fiber ratio, thus optimizing oxygen diffusion as previously described in the brain [24]. Erythropoietin also plays an important role in angiogenesis through upregulation of VEGF in ischemic rats [11]. Indeed, Wang et al. showed that neural progenitor cells treated with Epo were able to produce VEGF and consequently to promote angiogenesis through the upregulation of VEGFR2 expression in cerebral endothelial cells [70].

Our work provided novel physiological data about cerebral adaptations to chronic anemia. Indeed, we evidenced that Epo deficiency activated cerebral hypoxic mechanisms through HIF activation that promote angiogenesis [23]. In addition, the JAK/STAT signaling pathway mediated by the Epo/EpoR complex seems to be activated by chronic anemia [23, 47] and

could promote neuroprotection and cell proliferation [71]. Furthermore, more recent results showed that nNOS is specifically protective during anemia [57]. All these responses were probably able to minimize brain damage that could be induced by chronic anemia. Finally, the mechanisms responsible for matching capillary density to tissue oxygen levels are not unique to environmental hypoxic stimuli. Rather, these processes appear to be responsible for maintaining the oxygen availability through local blood flow in order to optimize the neuronal function.

3.2. Heart under chronic anemia

The classic physiological cardiovascular responses to anemia include an increased CO, a redistribution of blood flow and a decrease of hemoglobin-oxygen affinity. Two mechanisms are most probably responsible for the increased CO during anemia: reduced blood viscosity and increased sympathetic stimulation of the cardiovascular effectors. Blood viscosity affects both preload and afterload, two of the major determinants of the CO, whereas sympathetic stimulation primarily increases heart rate and contractility [72]. If cardiac function is normal, the increase in venous return or left ventricular preload will be the most important determinant of the increased CO during normovolemic anemia [72]. It is also known that anemia induces right and left ventricular hypertrophy [59, 73, 74] and increases CO, offsetting the fall in arterial oxygen content to maintain oxygen delivery. Our data confirmed the increase in CO by an increase in the stroke volume associated with a left ventricular dilatation as expected by Olivetti et al. [74]. Taken together, these data suggest that the enhancement in CO could be explained by both an increase in preload and autonomous nervous system stimulation. Indeed, our data showed an increase in myocardial function parameters in normoxic anemic mice. However, although the CO was increased in Epo-Tag^{h} mice, oxygen delivery remained lower than in controls. This could induce the stabilization of the transcription factor HIF-1α as already described in the brain in both models of acute and chronic anemia [61, 69]. This stabilization promotes VEGF-induced angiogenesis as shown in normoxic Epo-TAg^{h} mice with a rise in the capillaries/fibers ratio, thus optimizing oxygen diffusion as described in the brain [24]. This increase in capillary density could allow the development of cardiac hypertrophy without myocardial dysfunction, as previously described in rats in a model of anemia induced by iron-deficient diet [74]. Furthermore, we could not exclude that increased expression of nNOS also contributed to these adaptive cardiovascular responses in chronic anemic mice. Indeed, acute anemia resulted in an increase in CO and a reduced stroke volume in WT anemic mice while in contrast, CO and stroke volume responses were severely attenuated in anemic nNOS$^{-/-}$ mice [57]. In addition, a model of *Hif1a*$^{+/-}$ hemizigous mice revealed impaired increases in hematocrit, right ventricular mass and right ventricular pressure, allowing us to speculate that increased HIF-1α may have participated in these physiological responses to anemia in our model [75].

4. Effects of chronic anemia and hypoxia on cerebral and cardiac tissues

As previously explained, plethora of studies are available to describe cerebral and cardiac adaptations and their underlying molecular mechanisms in response to chronic hypoxia or

anemia separately. Our group investigated for the first time, the effects of chronic hypobaric hypoxia combined with chronic anemia in the heart and brain of the transgenic Epo-TAg^{h} mice. So far, the few studies from other groups that also use transgenic mice overexpressing Epo (Tg6 and Tg21) display results that are complementary to our data but also more detailed. Indeed, these studies also describe the pathways involved in the ventilatory responses to hypoxia and aim to clarify the role of Epo in respiratory acclimatization to hypoxia at physiological, cellular and molecular levels. Even though, we were not able to find other animal studies combining the effects of both chronic hypoxia and anemia on cardiac or cerebral tissues, comparing studies at a multidisciplinary level may provide new approaches and therapies for diseases associated with hypoxia.

4.1. Brain under chronic hypoxia and anemia

In the brain, both Epo and its receptor are upregulated during ischaemia/hypoxia [76, 77] and Epo administration considerably inhibits apoptosis after middle cerebral artery occlusion [78]. Apart from its positive effects in acute ischaemic brain damage, Epo is a potent stimulator of the hypoxic ventilatory response (HVR) by interacting with respiratory centers in the brainstem [79]. Indeed, the blockade of Epo's activity in the brainstem of adult C57Bl6 mice by intracisternal injections of the soluble Epo receptor (sEpoR) induced a reduction of the basal minute ventilation, but it did not affect the central chemosensitivity [80, 81]. In contrast, recent study using transgenic mice Tg6 (that present a human Epo gene overexpression in brain and circulation; Tg21: Epo overexpression in brain) suggested that Epo blunts the HVR through an interaction with central and peripheral respiratory chemocenters [81]. In our model, acute hypoxic ventilatory response was increased after chronic hypoxia in wild-type mice but remained unchanged in Epo-TAg^{h} mice, confirming that adequate erythropoietin level is necessary to obtain an appropriate HVR and a significant ventilatory acclimatization to hypoxia. Surprisingly, both constraints (chronic hypoxia and anemia) did not trigger a synergic effect in any studied parameters except a high cerebral NO level that could suggest an improved brain perfusion. Finally, the response to chronic hypoxia was divergent in the brain of wild-type and anemic mice. Indeed, these adaptation processes including angiogenesis and neuroprotection were globally altered in Epo-TAg^{h} mice exposed to chronic hypoxia.

Taken together, all these data suggest that Epo/EpoR pathways activation is necessary to initiate neuroprotection mechanisms as well as cerebral angiogenesis under hypoxia but also might help to better understand respiratory disorders at high altitude.

4.2. Heart under chronic hypoxia and anemia

Independently, chronic anemia and chronic hypoxia increased the expression of HIF-1α, VEGF and Epo, cytokines that are involved in both angiogenesis and cardioprotection through specific signaling pathways acting to compensate oxygen transport deficiency. Recent studies also involved these same cytokines in the cardiovascular responses as well as increased cardiac output observed in acute anemia [57, 75]. Our data showed a decrease in left ventricular hypertrophy and functional left ventricular adaptation as well as a reduced oxygen delivery in the heart of hypoxic Epo-TAg^{h} mice. Results from other groups showed that Tg6 mice did

not develop pulmonary hypertension in normoxia or after exposure to chronic hypoxia (10% O_2 for 3 weeks) [82] suggesting an important role of Epo in functional adaptation of the heart to chronic hypoxia. Similarly to what occurred in the brain, we did not observe a synergic effect of these combined constraints on the expression of the hypoxic genes in the heart of chronically hypoxic Epo-TAg^h mice suggesting that adaptive responses to both constraints are already maximal. However, the increased P-STAT-5/STAT-5 ratio is concordant with a direct protective effect of Epo on cardiomyocytes and endothelial cells as well as stimulation of angiogenesis in the ischaemic heart [83]. Capillary density was unchanged in spite of the fall in HIF-1α/VEGF pathway probably because the initiation of the capillarization with acute hypoxia necessitates VEGF, while its maintenance in chronic hypoxia involves other factors such as angiopoietins [38, 43].

Taken together, our results suggest that adaptative mechanisms that take place with chronic anemia are somewhat similar to those in response to 14 days of hypoxia. However, when both constraints are applied, these mechanisms failed to maintain an adequate cardiac adaptation with a secondary decrease in body oxygen supply, despite the activation of cardioprotective pathways.

5. Perspectives and significance

In this review, a proposal is made that chronic anemia-induced hypoxia triggers regulatory pathways that mediate long-term adaptive cardiac and cerebral changes, particularly at the transcriptional level. These adaptative mechanisms include a regulated increase in cerebral blood flow, cardiac output, angiogenesis and cytoprotection triggered by HIF-1α, VEGF and Epo pathways. All these compensatory mechanisms aim to optimize oxygen delivery and to protect the brain and heart from hypoxic injury to allow acclimatization. However, when both arterial pressure and content of oxygen are decreased, the cerebral and cardiac adaptative mechanisms showed their limitations. We could not exclude that cerebral and cardiac cell injuries occurred following the combined effect of chronic anemia and hypoxia as well as of the NO toxicity. **Figure 1** summarizes the cerebral and cardiac plasticity induced by chronic anemia and/or hypoxia. Data shown in this figure are all based on animal studies. Moreover, a recent review of our group includes also ventilatory [60], muscular [84, 85] and rheologic [86] adaptations in this model of mice. Finally, investigating the molecular mechanisms of O_2 homeostasis represents a mean of gaining new insights to the hypoxia-induced cerebral and myocardial injuries. But it is of great clinical importance to study extensively these non-erythropoietic functions of Epo to contribute to the development of new therapeutic strategies for the management of brain and heart ischemia.

Figure 1 summarizes the physiological adaptations to chronic hypoxia and anemia in the heart and brain of our model of Epo-TAg^h mice. The green color represents the responses of normoxic anemic mice. The blue color represents the responses of hypoxic control mice. The red color represents the responses of hypoxic anemic mice. The arrows represent an increase or decrease of the response, while the **'='** symbol means no change between normoxia and hypoxia. PaO_2 is arterial pressure of oxygen, CaO_2 is arterial content of oxygen, PiO_2 is inspired pressure of oxygen and TO_2 is transport of oxygen.

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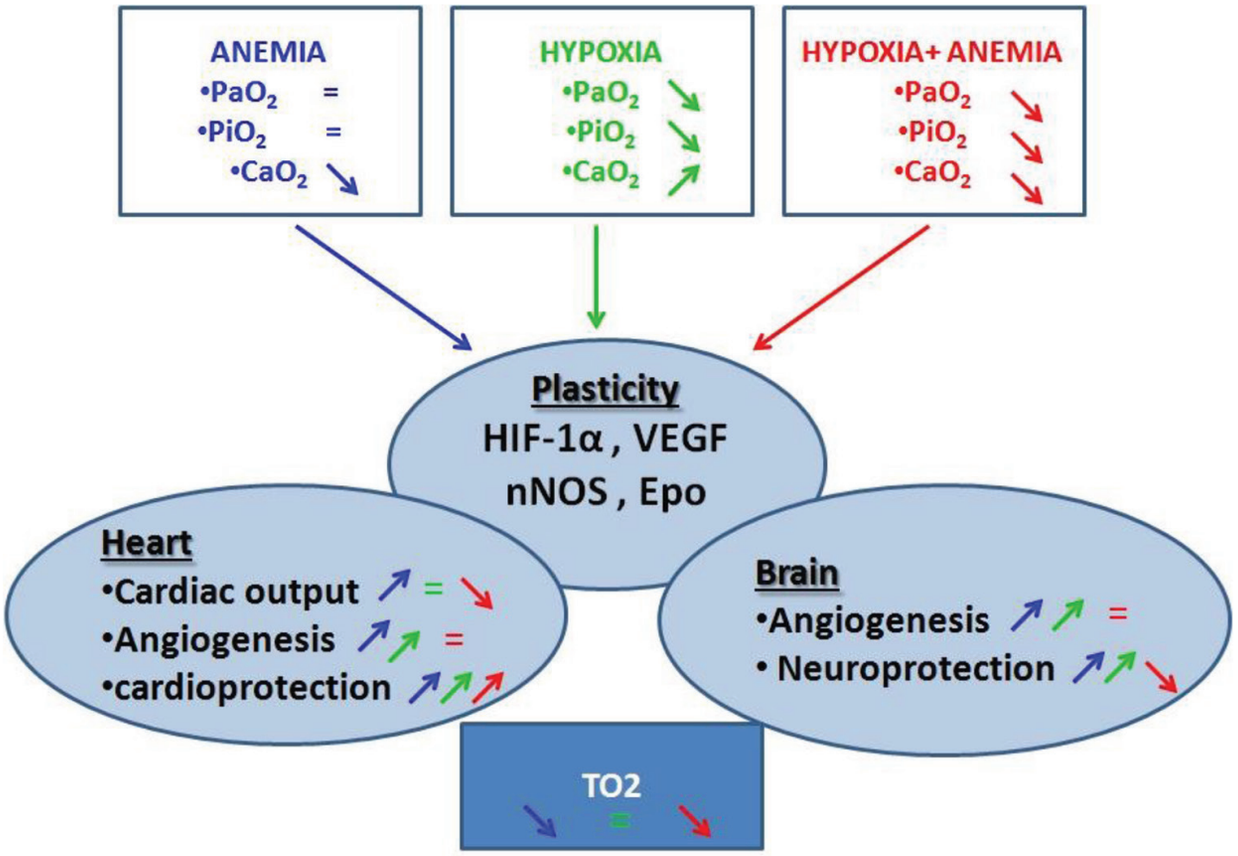

Figure 1. Cerebral and cardiac plasticity induced by chronic anemia and/or hypoxia in Epo-Tag^{h} mice.

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