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Research Report

# HIF-1α/COX-2 expression and mouse brain capillary remodeling during prolonged moderate hypoxia and subsequent re-oxygenation

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ABSTRACT

Dynamic microvascular remodeling maintains an optimal continuous supply of oxygen and nutrients to the brain to account for prolonged environmental variations. The objective of this study was to determine the relative time course of capillary regression during re-oxygenation after exposure to prolonged moderate hypoxia and expression of the primary signaling factors involved in the process. Four-month old male C57BL/6 mice were housed and maintained in a hypobaric chamber at 290 Torr (0.4 atm) for 21 days and allowed to recover at normoxia (room air) for up to 21 days. The mice were either decapitated or perfused in-situ and brain samples collected were either homogenized for Western blot analysis or fixed and embedded in paraffin for immunohistochemistry. Hypoxia inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF) and erythropoietin (EPO) expression were increased during hypoxic exposure and diminished during subsequent re-oxygenation. However, cyclooxygenase-2 (COX-2) and angiopoietin-2 (Ang-2) were both elevated during hypoxia as well as subsequent re-oxygenation. Significantly increased capillary density at the end of the 3rd week of hypoxia regressed back toward normoxic baseline as the duration of re-oxygenation continued. In conclusion, elevated COX-2 and Ang-2 expression during hypoxia where angiogenesis occurs and re-oxygenation, when micro-vessels regress, identifies these proteins as vascular remodeling molecules crucial for angioplasticity.



## 1. Introduction

The structural and functional integrity of the brain profoundly depends on a continuous and controlled supply of oxygen and glucose. The brain cannot tolerate extended periods of hypoxia or hyperoxia due to an insufficient energy supply to the brain by anaerobic glycolysis during hypoxia and production of excessive reactive oxygen specie (ROS) which causes damage to the genome, cellular contents, and membranes during hyperoxia (Bitterman, 2004; Fong, 2008). Therefore, transient vascular remodeling to regulate oxygen supply seems to be one of the major acclimatization

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mechanisms of the brain to fluctuations in tissue oxygen partial pressure (Dore-Duffy and LaManna, 2007; Pichiule and LaManna, 2002).

Prolonged exposure to moderate hypoxia produces a significant increase in cerebral capillary density in rats and mice alike (Benderro and Lamanna, 2011; Boero et al., 1999; Ndubuizu et al., 2010; Pichiule and LaManna, 2003; LaManna et al., 1992). The involvement of Ang-2 has been implicated in the reversibility of the angiogenic process, in the rat brain, when the animal was returned to normoxia (Pichiule and LaManna, 2003). However, whether HIF-1α and COX-2 protein levels were affected during re-oxygenation has not been recorded.

HIF is a critical mediator of endothelial growth factors and signaling proteins (such as VEGF, EPO, glycolytic enzymes and glucose transporters) during periods of metabolic stress and vascular remodeling (angiogenesis and regression) (Lum et al., 2007; Masson and Ratcliffe, 2003; Pichiule and LaManna, 2003; Semenza, 2004). Among the HIF isoforms, HIF-1α is thought to be the primary responder and main regulator of angiogenic changes during hypoxia due to its inductive effect on transcriptional activity of pro-angiogenic proteins such as VEGF (Semenza, 2004; Sharp and Bernaudin, 2004). Hypoxia is the main inducer of HIF-1α accumulation (Puchowicz et al., 2008; Semenza, 2007; Sharp and Bernaudin, 2004; Webb et al., 2009). Nevertheless, it was also shown that various metabolic and environmental stressors, which can disturb cellular homeostasis, can induce accumulation of HIF-1α under normoxic conditions (Benderro et al., 2012; Lu et al., 2002; Mekhail et al., 2004; Puchowicz et al., 2008).

On the other hand, in vitro and vivo results indicate COX-2 to be the main regulator of Ang-2 expression (LaManna et al., 2006; Pichiule et al., 2004). Recently, we reported HIF-1α and HIF-2α independent synergistic expressions of COX-2 and Ang-2 during chronic moderate hypobaric hypoxia and chronic moderate normobaric hyperoxia, in the mouse brain (Benderro and Lamanna, 2011; Benderro et al., 2012). Although increased expression of Ang-2 during re-oxygenation after chronic hypoxia was reported in the rat brain (Pichiule and LaManna, 2002), simultaneous relative expression of COX-2 was not determined.

Microvascular remodeling is one of the main mechanisms by which the brain acclimatizes to oxidative and metabolic stresses (Boero et al., 1999; Dore-Duffy and LaManna, 2007). In response to hypoxia, the increased capillary density results in decreased intercapillary diffusion distance thereby maintaining tissue oxygen tensions near normoxic levels (Boero et al., 1999; Fong, 2008; Harik et al., 1995). During prolonged hyperoxia appropriate avoidance response mechanisms are activated in which vascularity is diminished to regulate oxygen partial pressure in brain parenchyma, presumably to avoid excessive ROS (Benderro et al., 2012). Recently, we have deduced that brain capillary density is a continuously adjusted variable with tissue oxygen availability as one of the controlling modulators in this dynamic model of angioplasticity (Benderro et al., 2012).

In this study we have quantified changes in capillary density and relative time course of its regression during prolonged re-oxygenation after chronic moderate hypobaric hypoxia. We have also determined, for the first time, expression of HIF-1α, COX-2 and EPO proteins during re-oxygenation after acclimatization to chronic moderate hypoxia. Expressions of VEGF and Ang-2 were also determined and time-course changes in some physiological variables such as body weight, hematocrit and arterial oxygen saturation ($SaO_2$) were also measured.

Results indicate that decreased body weight and $SaO_2$, and increased hematocrit during hypoxia tended to return toward normoxic baseline during re-oxygenation. HIF-1α, VEGF and EPO protein expression, which were increased in hypoxia, diminished during re-oxygenation. However, COX-2 and Ang-2 expressions remained elevated during hypoxia as well as during re-oxygenation. Significantly increased capillary density at the end of the 3rd week of hypoxia regressed toward normoxic baseline when re-oxygenation period was prolonged.

## 2. Results

### 2.1. *Change in body weight during hypoxia and subsequent re-oxygenation*

All mice exposed to chronic hypoxia showed the expected rapid and significant ($p<0.05$) body weight loss during hypoxic exposure (Fig. 1). Average normoxic body weight (29.2±3.9 g) decreased to 22.9±3.3 g at 4th day of hypoxia. Then, a slight recovery was observed on the 7th, 14th, and 21st (24.4±3.8 g) day of hypoxia. The lost body weight was regained rapidly after re-oxygenation. The average body weight during re-oxygenation increased from 26.6±3.4 g on the 1st day to 29.9±3.7 g on the 21st day of re-oxygenation.

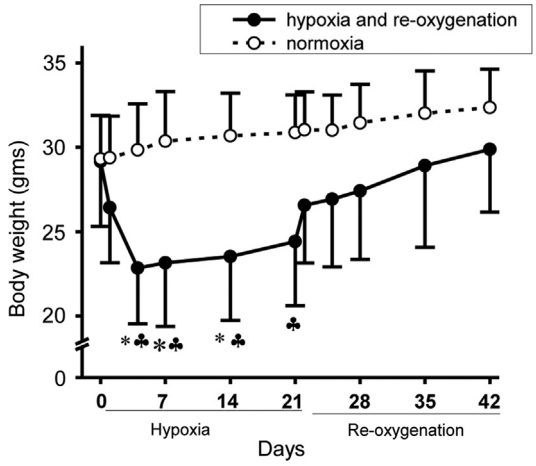


**Fig. 1 – Change in body weight in 4-month old mice during hypoxia and recovery from hypoxia (re-oxygenation). Solid lines indicate change in body weight during 21 days of hypoxia followed by 21 days of re-oxygenation. Dashed lines indicate weight change in normoxic littermate control during the experimental periods. $^{*}P<0.05$ compared with initial normoxic value. $^{\clubsuit}P<0.05$ compared with corresponding normoxic value. Values are mean±SD; $n=16$ for normoxia; 10 for 1, 4, 7, 14, and 21 days of hypoxia and day 1 re-oxygenation; 9 for 4, 7, 14 days of re-oxygenation and 5 for 21 days of re-oxygenation.**