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Research Report

Hypoxia-inducible factor-1 (HIF-1)-independent microvascular angiogenesis in the aged rat brain

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ABSTRACT

Angiogenesis is a critical component of mammalian brain adaptation to prolonged hypoxia. Hypoxia-induced angiogenesis is mediated by hypoxia-inducible factor-1 (HIF-1)-dependent transcriptional activation of growth factors, such as vascular endothelial growth factor (VEGF). Microvascular angiogenesis occurs over a 3-week period in the rodent brain. We have recently reported that HIF-1α accumulation and transcriptional activation of HIF target genes in the aged cortex of 24-month-old F344 rats is significantly attenuated during acute hypoxic exposure. In the present study, we show that cortical HIF-1α accumulation and HIF-1 activation remain absent during chronic hypoxic exposure in the aged rat brain (24-month-old F344). Despite this lack of HIF-1 activation, there is no significant difference in baseline or post-hypoxic brain capillary density counts between the young (3-month-old F344) and old age groups. VEGF mRNA and protein levels are significantly elevated in the aged cortex despite the lack of HIF-1 activation. Other HIF-independent mediators of hypoxia-inducible genes could be involved during chronic hypoxia in the aged brain. PPAR-γ coactivator (PGC)-1α, a known regulator of VEGF gene transcription, is elevated in the young and aged cortex during the chronic hypoxic exposure. Overall, our results suggest a compensatory HIF-1-independent preservation of hypoxic-induced microvascular angiogenesis in the aged rat brain.

1. Introduction

The mammalian brain naturally functions in a low, but controlled, oxygen environment and is particularly sensitive to alterations in oxygen delivery (LaManna et al., 2004; Ndubuizu and LaManna, 2007; Sick et al., 1982; Silver and Erecinska, 1998). Metabolic demand and insufficient energy storage make the brain vitally dependent on a constant supply of oxygen and nutrients to allow adequate oxidative phosphorylation and ATP production. Metabolic stressors, such as tissue hypoxia, trigger compensatory mechanisms in attempt to restore the balance between local oxygen delivery and tissue oxygen consumption (Dunn et al., 2000; LaManna et al., 2004). A more detailed review of this has been described in LaManna et al. (2004). Immediate systemic responses to acute imbalances in oxygen delivery include increased ventilation, cardiac output, and cerebral blood flow. Days later, an increase in red blood cells aids in the attempt to restore oxygen tension. If the tissue hypoxia persists, mechanisms that promote angiogenesis, the formation of new blood vessels from preexisting vasculature, are activated thus increasing vascular density. In the rodent brain, adaptive microvascular angio-

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genesis is a multifaceted process requiring an orchestrated sequence of events and occurs over a 3-week period (Boero et al., 1999; Dunn et al., 2000; LaManna, 1992; Pichiule and LaManna, 2002; Ward et al., 2007). The end result of this response to chronic hypoxia is a decrease of intercapillary diffusional distances and normalization of tissue oxygen tension (Dunn et al., 2000; LaManna, 1992).

Angiogenesis is a tightly regulated process that involves the proliferation, migration, differentiation, and organization of endothelial cells into new functional microvessels (Fong, 2008; LaManna et al., 2004; Pugh and Ratcliffe, 2003b). These mature microvessels, consisting of endothelial cells and adjacent pericytes, maintain a special structural relationship with glial astrocytes, neurons, and the surrounding extracellular matrix called the neurovascular unit (NVU) (LaManna, 1992; LaManna et al., 2004). Angiogenesis is an adaptive response to tissue hypoxia in a number of *in vivo* and *in vitro* models, including development and tumor models (Pugh and Ratcliffe, 2003a; Semenza, 2000b,c, 2007b). A key mediator of this angiogenic response is hypoxia-inducible factor-1 (HIF-1), which is responsible for the transcriptional activation of a number of growth factors such as vascular endothelial growth factor (VEGF) (Semenza et al., 1997; Semenza, 2000a; Semenza, 2001a,b, 2007a). Secreted VEGF, from astrocytes and pericytes, bind to VEGF receptors on the surface of endothelial cells activating receptor tyrosine kinases. HIF-1 has also been shown to have an essential role in the development of systemic vessels and other brain cell types during embryogenesis with HIF-1 knockouts being embryonic lethals by E10–11 primarily due to circulatory system defects (Semenza, 2001a; Sharp and Bernaudin, 2004). These embryonic mice have also shown evidence of inadequate brain development likely due to impaired vascular development as well as massive cell death in the cephalic mesenchyme (Acker and Acker, 2004; Fong, 2008; Iyer et al., 1998; Park et al., 2003; Ryan et al., 1998).

We have recently reported an age-dependent decline in cortical HIF-1α accumulation and transcriptional activation of HIF target genes in response to 72 hours of hypobaric hypoxic exposure (Ndubuizu et al., 2009). In comparison to the response of younger Fischer 344 (F344) rats (3–12 months of age), the induction of cortical HIF-1α accumulation was completely attenuated in old F344 rats (24 months) following hypoxia and comparable to that of age-matched controls. Hypoxic-induced upregulation of HIF target gene mRNA, such as VEGF, was also completely attenuated in the aged cortex. This phenomenon appears to be post-translational with mRNA expression of HIF-1α intact in the aged cortex. This attenuated HIF-1α response was directly correlated with an increase in the cortical expression of the HIF-regulatory enzymes, prolyl hydroxylase domain (PHD)-containing proteins, in the aged rats relative to their younger counterparts. Attenuation of HIF-1α accumulation and transcriptional activation of growth factors such as VEGF might impair angiogenic responses in the brain of the aged rat. Diminished compensation for insufficient tissue oxygenation could make senescent rats more susceptible to ischemia and chronic hypoxic. This attenuated response might result in failure to maintain normal microvascular density, thus potentially affecting neuronal survival and plasticity-associated learning.

In this study, we examined microvascular angiogenesis in the aged F344 rat brain following three weeks of chronic hypobaric hypoxia and have compared it to that of younger (3 months) F344 rats. Multiregion analysis of the brain, including parietal cortex, corpus callosum, striatum, and CA1 region of the hippocampus, was done to compare the microvascular angiogenic response of the young and aged rodent brain in response to prolonged hypoxia. Our results showed no significant difference in baseline capillary density between the young and aged rats in any of the four regions. The adaptive increase in brain microvasculature following 3 weeks of hypoxia is still intact in the aged brain and as robust as in the young rat brain. The increase in brain microvasculature is comparable to that of young rats and occurred despite the persistent attenuation of HIF-1α accumulation during the chronic hypoxic exposure. Although our previous study showed a lack of VEGF mRNA upregulation in the aged cortex following a 3-day hypoxic exposure, there appears to be a delayed increase in cortical mRNA and protein expression of VEGF in the aged cortex during chronic hypoxic exposure. The robust VEGF mRNA expression in the aged cortex suggests other mechanisms of transcriptional regulation that remain intact in the aged rats. PPAR-γ coactivator (PGC)-1α, a known regulator of VEGF gene transcription and angiogenesis, is elevated in the young and aged cortex during the chronic hypoxic exposure. This demonstrates HIF-independent regulation of VEGF and angiogenesis that is maintained in the aged rat cortex.

2. Results

2.1. *Systemic physiological changes*

The young and old F344 rats showed a significant elevation of blood hematocrit following chronic hypoxia relative to controls (increase from 50±2.8% to 78±2% in and 46±2.2% to 75±3.4% in the young and old rats, respectively, Fig. 1A). Overall, there were 56% and 64% increase in blood hematocrit on the young and old rats, respectively. Despite a difference in control body weight (292±23 g in the young versus 405±30 g in the old), both age groups displayed similar trends in body weight decline during the 3-week hypoxic exposure (Fig. 1B). In both age groups, there was a decline in body weight over the first 2 weeks that stabilized by the third week. The maximal percent decline in body weight was 20% in both age groups.

2.2. *Increase in cortical microvascular density following hypoxia as a function of age*

Cortical cerebral microvessels were identified by counting the number of positive capillaries per unit area. Fig. 2A shows representative photomicrographs of GLUT-1-stained sections in the parietal cortex of young and old F344 rats following 3 weeks of chronic hypoxia relative to controls. The capillary density increase as a function of age is shown in Fig. 2B. Following hypoxic exposure, there was a 41% and 48% increase in cortical microvessels in the young and old rats, respectively. There was an average increase in cortical microvessels from 455±47 to 642±35 in the young and from 408±21 to 603±45 in