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Research Report

Activin A regulation under global hypoxia in developing mouse brain

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ABSTRACT

Activin A is a multifunctional growth and differentiation factor with pronounced neuroprotective properties that is strongly up-regulated in various forms of acute brain disorders and injuries including epilepsy, stroke and trauma. In a pediatric context, activin A has been advanced as a potential marker for the severity of perinatal hypoxic–ischemic brain injury. Here we investigated the regulation of activin A under global hypoxia without ischemia in primary cultures of cortical neurons and in neonatal and adult mice of two strains (C57BL/6 and CD-1). From birth to adulthood, activin βA subunit, activin receptors, and functional activin antagonists were all expressed at roughly similar mRNA levels in the brain of C57BL/6 mice. Independent of mouse line and age, we found both moderate (11% O_2, 2 h) and severe hypoxia (8%, 6 h) to be consistently associated with normal or even reduced levels of activin βA (*Inhba*) mRNA. The surprising unresponsiveness of *Inhba* expression to hypoxia was confirmed at the protein level. In situ hybridization did not indicate regional, hypoxia-related differences in *Inhba* expression. Pharmacologic stabilization of hypoxia inducible factors with the prolyl hydroxylase inhibitor FG-4497 did not influence *Inhba* mRNA levels in neonatal mice. Our data indicate that pure hypoxia differs from other, more complex types of brain damage in that it appears not to recruit activin A as an endogenous neuroprotective agent.

1. Introduction

Activin A is a multifunctional growth and differentiation factor of the transforming growth factor-β (TGF-β) family. In the brain, activin A receives increasing attention as a potent neurotrophic and protective factor and a master regulator of synaptic transmission and plasticity (reviewed in Krieglstein et al., 2011). Structurally, activins are disulfide-linked homo- or

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hetero-dimers composed of two β chains, with activin A (βA/βA), activin AB (βA/βB), and activin B (βB/βB) being the best-characterized variants. Heteromeric receptor complexes consisting of type II (ActRIIA, ActRIIB) and type I receptors (ActRIB, ActRIC) mediate the biological activities of activin. Follistatin and inhibins are functional antagonists of activin A. The secreted glycoprotein follistatin binds to activin and prevents it from binding to its receptors (McDowall et al., 2008). Inhibins are heterodimers of one α subunit combined with one βA or one βB subunit and compete with activin for receptor binding (Zhu et al., 2012).

The level of activin A in brain tissue is normally low, but fast and transient up-regulation has been observed under physiological conditions, e.g. after high frequency neuronal activity causing synaptic plasticity, and, much more so, under pathophysiological conditions leading to tissue damage (Ageta and Tsuchida, 2011; Krieglstein et al., 2011). In rodents, hypoxic–ischemic insults provoked by middle cerebral artery occlusion led to strong up-regulation of activin βA (*Inhba*) mRNA levels in the adult (Lai et al., 1996; Bottner et al., 2006; Mukerji et al., 2007) and developing brain (Wu et al., 1999). A similar increase in *Inhba* mRNA and in activin A protein was observed after intracerebroventricular (icv.) kainate injection mimicking excitotoxic brain damage (Inokuchi et al., 1996; Tretter et al., 1996, 2000). Elevated activin A levels are functionally relevant, since icv. injection of recombinant activin A promoted neuronal survival and attenuated cerebral damage in animal models of stroke and excitotoxic damage (Wu et al., 1999; Tretter et al., 2000; Mukerji et al., 2007). Vice versa, blockade of activin signaling by recombinant follistatin or over-expression of dominant negative ActRIB counteracted the protective effect (Tretter et al., 2000; Müller et al., 2006).

Activin has also been implicated in injuries of the neonatal brain. In asphyxiated full-term newborns, enhanced activin A concentrations were detected in their cerebrospinal fluid, plasma and urine, and the degree of elevation was related to the severity of hypoxic–ischemic encephalopathy (Florio et al., 2003; Florio et al., 2004, 2007; Fiala et al., 2012), but see (Tong, 2006).

Whereas the regulation of activin A expression in asphyxia, stroke and other complex injuries has been well documented, it remains to be determined how severe hypoxia alone affects the levels of activin A in the neonatal and adult brain. Here, we systematically examined the developmental, temporal and spatial expression patterns of activin A during global hypoxia in the immature and adult mouse brain, using an established model of acute systemic hypoxia (Trollmann et al., 2008; Hümmler et al., 2012). We report the unexpected finding that, in contrast to other types of brain injury, hypoxia alone did not cause an increase in activin A.

2. Results

2.1. *Hypoxia does not affect Inhba mRNA level in primary cortical neurons*

Primary embryonic neuronal cell cultures of E14 C57BL/6 mouse cortex were exposed to severe hypoxia (1% O_2, 4 h) at DIV6. RT-qPCR showed that the activin/inhibin βA gene (*Inhba*) (Fig. 1A), activin receptors ActRIIA, ActRIIB, and ActRIB (data not shown) were expressed in cultured neurons and did not change significantly in response to hypoxia (1% O_2). To verify the effectiveness of the hypoxia model, we measured mRNA levels of vascular endothelial growth factor A (*Vegfa*). Consistent with previous publications (Trollmann et al., 2003, 2008; Fandrey and Gassmann, 2009; Zhang et al., 2009), *Vegfa* mRNA was significantly up-regulated ($P<0.05$) during hypoxia compared to normoxia (Fig. 1B).

2.2. *Expression of the activin βA subunit, functional antagonists and receptors during mouse brain maturation*

Given the limitations of cell culture models, we turned to in vivo experiments to further explore the effects of hypoxia on activin. As a prelude to these experiments, we investigated the expression of *Inhba*, activin receptors, and functional activin antagonists (*Inha, Fst*) at different stages of postnatal development. Assessments were performed using C57BL/6 mouse brains from postnatal days P0, P3, P10, P14, P21, and P60. Using RT-qPCR, we found generally stable expression of activin receptors during the first 8 weeks of life, although expression of *ActRIB* increased 1.5-fold during this time period ($P<0.05$). Expression of *Inhba* did not show

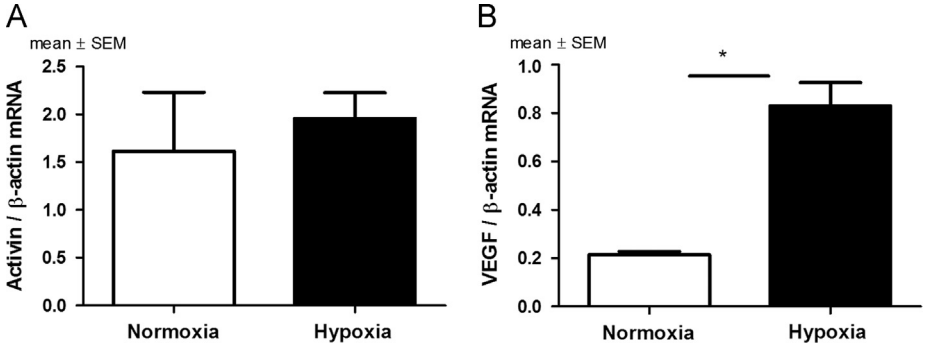

Fig. 1 – Expression of the activin βA subunit (*Inhba*) and *Vegfa* in primary cortical mouse neurons at DIV6 (DIV: days in vitro) under normoxic and hypoxic conditions determined by RT-qPCR. (A) *Inhba* expression remained unchanged while (B) *Vegfa* was upregulated significantly. Expression of β-actin was used for normalization. Use of porphobilinogen deaminase expression for normalization showed similar results ($n=3$; $^*P<0.05$).