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Prenatal hypoxia may aggravate the cognitive impairment and Alzheimer's disease neuropathology in $APP^{Swe}/PS1^{A246E}$ transgenic mice

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Abstract

Most cases of Alzheimer's disease (AD) arise through interactions between genetic and environmental factors. It is believed that hypoxia is an important environmental factor influencing the development of AD. Our group has previously demonstrated that hypoxia increased β-amyloid (Aβ) generation in aged AD mice. Here, we further investigate the pathological role of prenatal hypoxia in AD. We exposed the pregnant $APP^{Swe}/PS1^{A246E}$ transgenic mice to high-altitude hypoxia in a hypobaric chamber during days 7–20 of gestation. We found that prenatal hypoxic mice exhibited a remarkable deficit in spatial learning and memory and a significant decrease in synapses. We also documented a significantly higher level of amyloid precursor protein, lower level of the Aβ-degrading enzyme neprilysin, and increased Aβ accumulation in the brain of prenatal hypoxic mice. Finally, we demonstrated striking neuropathologic changes in prenatal hypoxic AD mice, showing increased phosphorylation of tau, decreased hypoxia-induced factor, and enhanced activation of astrocytes and microglia. These data suggest that although the characteristic features of AD appear later in life, hypoxemia in the prenatal stage may contribute to the pathogenesis of the disease, supporting the notion that environmental factors can trigger or aggravate AD.

Keywords: Alzheimer's disease; Prenatal hypoxia; Learning and memory; Synapses; β-amyloid; Tau protein; Neprilysin; Hypoxia-inducible factor

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease characterized by cognitive impairment and dementia (Goedert and Spillantini, 2006). Many AD cases are sporadic, which means they have no defined cause. Environmental factors and genetic susceptibility are most commonly considered to play an important role in sporadic cases (Mayeux, 1998; Ryman and Lamb, 2006; Vemuri et al., 2010). Patients with cerebral infarction showed detrimental cognitive impairment (Heyman et al., 1998), suggesting that reduced cerebral perfusion leading to hypoxia in cerebral vascular diseases is an important AD contributor. A recent clinical study has demonstrated that older women with sleep-disordered breathing and hypoxia had an increased risk of developing cognitive impairment and dementia (Yaffe et al., 2011). Further research has found that hypoxia resulting from cardiac arrest may lead to a time-dependent increase in serum β-amyloid (Aβ) peptide level (Zetterberg et al., 2011), implying that hypoxia may contribute to AD pathogenesis (Oresic et al., 2011). In the AD model, hypoxia exposure has been found to increase Aβ deposition and neuritic plaque formation, and potentiate the memory deficit (Sun et al., 2006). Our previous study also

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showed that repeated hypoxia aggravates the pathologies of AD in aged $APP^{Swe}/PS1^{A246E}$ transgenic ($Tg^{APP/PS1}$) mice (Li et al., 2009). These studies indicate that hypoxia may play an important role in AD pathogenesis.

The fetus may experience hypoxic stress under a variety of conditions, such as pregnancy at high altitude and pre-eclampsia (Patterson et al., 2010). Some reports showed that a single prenatal hypoxic precondition could induce neuroprotection in the ischemic animal model (Zhao and Zuo, 2005), and that multigenerational high altitude residents are more protected from the altitude-associated reduction in uterine artery blood flow than shorter-term residents (Moore et al., 2011). In contrast, many animal studies have indicated that prenatal hypoxia could increase the risk of cardiovascular disease and pulmonary disease (Li et al., 2004; Morton et al., 2011; Patterson et al., 2010). A new report showed that living at high altitudes may have a harmful effect on chronic obstructive pulmonary disease (Ezzati et al., 2012). Moreover, throughout the gestation period, pregnant woman with pre-eclampsia often suffer from intrauterine chronic intermittent hypoxia. Intermittent rather than sustained long-lasting hypoxia is more likely to occur during fetal life. A typical example is the occurrence of obstructive sleep apnea in pregnant woman (Gozal et al., 2003). Therefore, we propose that prenatal chronic intermittent hypoxia may affect the development of AD in adult life. However, the effects of prenatal hypoxia on the memory and neuron function of adults are not known at this time. Previous studies reported that prenatal hypoxia could cause impairment of cognitive functions in the postnatal period (Golan and Huleihel, 2006; Salchner et al., 2003), and could result in neurodegeneration, glial proliferation, and neurotransmitter changes (Bernert et al., 2003; Northington et al., 2001). Therefore, we propose that prenatal hypoxia may provoke long-term detrimental effects on the central nervous system, which may contribute to the pathogenesis of AD.

The characteristic senile plaques of AD are primarily composed of 39–43 Aβ peptide derived from amyloid precursor protein (APP). It has been reported that APP level are elevated for a long period of time following mild and severe ischemia in the human brain (Pluta et al., 1998). Focal ischemic insults or chronic hyperfusion in ischemic animal models can lead to increased accumulation of APP and enhanced Aβ peptide generation (Stephenson et al., 1992). The offspring of rats subjected to hypoxia at 13–14 days of gestation exhibit high level of APP during every period of life (Nalivaeva et al., 2003). These results indicate that hypoxia may influence the expression, regulation, and processing of APP, and may potentiate the course of amyloidogenesis during adult life.

In sporadic AD, which comprises more than 90% of AD, the accumulation of Aβ peptide might be mainly attributed to a deceleration of Aβ peptide degradation (Chételat et al., 2011). Neprilysin (NEP) is identified as a major Aβ-degrading enzyme in the brain (Maruyama et al., 2005; Spencer et al., 2008). Conversely, reduced NEP expression results in Aβ peptide accumulation in synapses and causes learning and memory abnormalities (Farris et al., 2007; Huang et al., 2006). These findings outline the potentially important role of NEP in AD. We recently found that NEP is downregulated by histone modification in hypoxia-treated mouse primary neurons (Wang et al., 2011).

In the present study, we used $Tg^{APP/PS1}$ mice to study the role of prenatal hypoxia in AD development in adults. We found that prenatal chronic intermittent hypoxia significantly potentiates memory deficit, and exacerbates their AD-related neuropathologies. Our study indicates that prenatal hypoxia is a risk factor that may aggravate the development of AD in adults.

2. Methods

2.1. Animals and hypoxic treatment

Thirty-six time-mated pregnant transgenic mice (the Jackson Lab, no. 003378, $Tg^{APP/PS1}$) were assigned randomly to hypoxic and normoxic groups (18 mice in each group). Hypoxic mice were exposed to simulated high-altitude hypoxia in a hypobaric chamber (Jinyu Bio-Technology Co., Ltd., Shanghai, China) mimicking 5000 m in altitude (oxygen, 11.1%) for 6 hours per day for days 7–20 of gestation. Normoxic mice were maintained in a similar chamber with normobaric conditions. Six hours later, all the mice were returned to animal housing cages. The pups were all delivered under normobaric conditions. At 1 month old, the genotypes of these mice were analyzed by polymerase chain reaction analysis of genomic DNA from tail biopsies (Huang et al., 2011). A total of 144 mice were used for the whole experiment at 3 different observation times (mice at 3, 6, and 9 months of age). We divided the mice into the following 4 groups: wild type (Wt) mice with prenatal normoxic treatment (Wt+N; $n = 33$) or hypoxia treatment (Wt+H, $n = 36$), transgenic (Tg) mice with prenatal normoxic treatment (Tg+N, $n = 36$) or hypoxic treatment (Tg+H, $n = 39$). Mice at the age of 3 months ($n = 46$), 6 months ($n = 48$), or 9 months ($n = 50$) performed learning and memory tests before being sacrificed and then electron microscopy examination, neuropathology detection, and neurobiochemistry assays were performed. Animal care and procedures were performed in accordance with the Laboratory Animal Care Guidelines approved by Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences.

2.2. Morris water maze tests

We carried out the modified Morris water maze test (Morris, 1984; Zhang et al., 2008). The procedures consisted of 1 day of the visible platform test and 4 days of the