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Hippocampal NMDAR-Wnt-Catenin signaling disrupted with cognitive deficits in adolescent offspring exposed to prenatal hypoxia

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ABSTRACT

Prenatal hypoxia (PH) is one of the most common stresses on fetuses, and might lead to abnormal brain development. This work investigates whether PH affects behavioral development of the learning/memory ability in the adolescent offspring rats and the underlying molecular basis in the brain. In this study, pregnant rats used to generate PH offspring were treated with hypoxia (10.5% oxygen) from gestational day 4 to 21. Brain weights of either the fetuses or the 6-week old offspring in the PH group were found to be significantly lower compared with the control group. Morris water maze tests showed longer escape latency and swimming distance during navigation testing in the PH offspring; retention tests demonstrated less frequency of crossing target areas indicating impaired learning and memory ability in the PH offspring. The expressions of subunits of N-methyl-D-aspartate receptors (NMDARs), *Grin1*/NR1, *Grin2a*/NR2A, and *Grin2b*/NR2B, were significantly decreased in the hippocampus of adolescent offspring in the PH group. Wnt3a as well as active form of β-catenin protein were also significantly down-regulated. Furthermore, the expression of early response gene, *Fosl1*, was significantly reduced. The results above provide new evidence that PH might result in the spatial acquisition and retrieval deficits in the adolescent offspring, associated with dysregulation of NMDARs-Wnt-Catenin signaling in the hippocampus. This study result deepens the knowledge of the long-term influence of prenatal insults on the neuro-behavioral development.

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1. Introduction

The hypothesis on developmental origins of health and disease (DOHaD) or fetal origins of adult diseases (FOAD) associates intrauterine and maternal stresses with high risk of diseases in postnatal life, including cancers, cardiovascular diseases, and neurologic diseases (Barker, 1990; Saffery, 2014). Animal studies and human investigations indicate that prenatal stresses could reprogram hippocampal functions and affect offspring behaviors in late life (Postma et al., 2013; Barzegar et al., 2015; Depino, 2015). Hypoxia is one of the most common clinically relevant stresses on fetuses and the major cause of brain injury for the newborns (Regnault et al., 2007; Miller et al., 2015). In fact, many conditions resulting in fetal hypoxia, such as ischemic-hypoxic conditions, high altitude, preeclampsia, and smoking, have been associated with intrauterine growth restriction (IUGR), which not only increases the risk of perinatal mortality and morbidity, but also causes neuro-developmental delay in childhood (Hickey et al., 1978; Cogswell and Yip, 1995; Getahun et al., 2013). More and more evidences show that intrauterine hypoxia affects brain development and results in mild behavioral dysfunctions, severe seizures, mental retardation, and even fetal death (Mach et al., 2009). Furthermore, prenatal exposure to hypoxia is considered to be associated with attention-deficit/hyperactivity disorder (ADHD) and autism in childhood, a critical period for intelligence development (Froehlich-Santino et al., 2014; Miguel et al., 2015). This study proposes that prenatal hypoxia (PH) might induce long-term modifications on gene and protein expression related to intelligence development in the adolescents. Since the underlying molecular basis in the brain is unclear yet, the present study focuses on solving this issue.

Long-term potentiation (LTP) is an activity-dependent form of synaptic plasticity. It is considered to play a critical role in the learning and memory formation (Lynch, 2004). N-methyl-D-aspartate receptors (NMDARs) are the predominant molecules in control of synaptic plasticity, memory functions, and maintaining LTP (Nakazawa et al., 2004). NMDARs are tetrameric protein complexes usually comprising of two NR1 and two NR2 subunits (Kalbaugh et al., 2009). NR1 subunits are located in a site important for synaptic trafficking, potentially favoring synaptic receptor retention (Korshunova et al., 2015). Among four types of NR2 subunits (NR2A-D), NR2A and NR2B are predominantly expressed in the hippocampus and cortex, and play distinct roles in long-term synaptic plasticity (Erreger et al., 2005). Hypoxia at postnatal day 10 results in long-term impaired spatial learning and memory, and is associated with reduced expression of NMDAR subunits (Chen et al., 2007). However, it is still unknown whether NMDARs could be modified by hypoxia during pregnancy with possibly impaired learning and memory in the adolescent offspring.

Other than NMDAR, recent studies have shown that the Wnt pathway is critical in synaptic transmission and activity-dependent synaptic plasticity, and plays important roles in positively regulating LTP with its activation or suppression (Chen et al., 2006). Wnts are highly conserved glycoproteins that bind to several distinct receptors and activate multiple downstream signaling pathways (Reiner and Sapir, 2005). When Wnts bind to the Frizzled (Fz) and low-density lipoprotein-related protein (LRP) receptors, the cytoplasmic protein Disheveled (Dvl) is recruited to the membrane, inducing the degradation of glycogen synthase kinase-3 (GSK-3) and the activation of β-catenin. Afterwards, activated β-catenin translocates into the nucleus and forms a complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors, hence regulates the expression of downstream target genes (MacDonald et al., 2009). Wnt/β-catenin pathway is active in the hippocampal neurogenic niche and functions as a principal regulator of hippocampal neurogenesis (Lie et al., 2005). Deregulated Wnt signaling has been implicated in the pathology of Alzheimer's Disease (AD) (Inestrosa and Toledo, 2008), which is characterized by progressive memory loss and cognitive impairment, strongly suggesting a role for Wnt/β-catenin signaling in learning and memory processes. It has been demonstrated that oxygen regulated proliferation of neural stem cells through Wnt/β-catenin pathway (Braunschweig et al., 2015). It is unknown whether Wnt/β-catenin signaling is involved in the long-term effect on hippocampal neurogenesis in the adolescence offspring exposed to PH. Therefore, such signaling in the offspring hippocampus as well as learning behavior following PH is studied in the present study.

2. Results

2.1. *Reduced brain and body weight in the offspring exposed to prenatal hypoxia*

PH significantly decreased brain weight of the fetuses at GD21 ($P<0.05$) (Fig. 1A) and the offspring 6 weeks after birth ($P<0.01$) (Fig. 1C). Body weight in the PH group was lower at GD21 ($P<0.05$) (Fig. 1B); however, adolescent body weight in the PH group was not significantly different ($P>0.05$) (Fig. 1D).

2.2. *Spatial learning and memory deficits in the adolescent offspring exposed to prenatal hypoxia*

Morris water maze was conducted to assess whether PH has long-term effect on the spatial learning and memory ability of the offspring rats at adolescent age (6 weeks). During the trial, both groups of animals improved their performance and found the platform with a decreasing escape latency and travel distance (Fig. 2). On day 1, there was a difference in the escape latency between the PH group and the control group ($P<0.05$). From day 2 to day 4, the difference in the escape latency between the two groups became significant ($P<0.001$), while no significant difference was observed on day 6 ($P>0.05$) (Fig. 2A). Travel distance was also found to be shortened for both groups animals through the 7-day training period. On day 1, there is a noticeable difference in path length between the PH group and the control group ($P<0.001$). However, this difference became less significant from day 2 to day 4 ($P<0.05$), and no significant difference was observed on the day 5 ($P>0.05$) (Fig. 2B). There was no significant change found with swim speed between two groups (Fig. 2C). On the last day (Day 8), the probe trail was conducted. Compared