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Research Report

Effects of chronic hypoxia in developing rats on dendritic morphology of the CA1 subarea of the hippocampus and on fear-potentiated startle

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ABSTRACT

Chronic hypoxia (CH) present in infants with cyanotic congenital heart disease may be responsible for subsequent cognitive deficits seen in these children. In a rat model of CH [10% O_2 between postnatal day (P) 3 and 28], we have demonstrated significant alterations in energy metabolism and excitatory neurotransmission in the developing hippocampus. These alterations may adversely affect dendritic morphology, which is a highly energy-dependent and excitatory neurotransmitter-mediated event, and hippocampus-mediated behaviors. We measured the apical segment length of dendrites in pyramidal neurons of the CA1 region of the hippocampus using microtubule-associated protein-2 (MAP-2) histochemistry on P28 while the animals were hypoxic (n=8 in CH and n=6 in control), and on P56 after the animals had been normoxic for 4 weeks (n=8/group). We also compared dorsal hippocampus-dependent trace fear conditioning and dorsal hippocampus-independent delay fear conditioning on P56. Developmental trajectory of the apical segment length was similar in CH and controls, decreasing between P28 and P56. However, when compared with the controls, the apical segment length was longer in the CH group on both P28 [55.11±2.30 μm (CH) vs. 40.52±1.20 μm (control), p<0.001] and P56 [44.01±1.56 μm (CH) vs. 31.75±1.31 μm (control), p<0.001], suggesting the persistence of an immature dendritic architecture. Both trace and delay fear conditioning were decreased in the CH group, suggesting functional abnormality beyond the dorsal hippocampus. These structural and functional alterations may contribute to the cognitive deficits seen in infants at risk for CH.

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Abbreviations: CAMK-II, calcium calmodullin kinase II; CH, chronic hypoxia; Cr, creatine; MAP-2, microtubule-associated protein-2; PBS, phosphate buffered saline; PCr, phosphocreatine; P, postnatal day; CS, conditioned stimulus; US, unconditioned stimulus

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1. Introduction

The incidence of cyanotic congenital heart disease is approximately 2 per 1000 live births (Hoffman, 2000). Advances in the techniques of cardiopulmonary bypass and hypothermia have improved the survival rates of these infants (Mayer, 1998). Central nervous system injury is a major cause of neurological morbidity in infants with cyanotic congenital heart disease (Wray and Sensky, 1999). These infants are exposed to multiple risk factors, such as ischemia, infection, and acute and chronic hypoxia, of which chronic hypoxia (CH) may play a significant role in the neurodevelopmental deficits.

Children with cyanotic congenital heart disease are in a state of CH during the first 2–3 years of life prior to the repair of their cardiac lesion. This period of CH encompasses the phase of rapid hippocampal growth, where neuronal proliferation, migration, glial cell proliferation, synaptogenesis, and myelination are ongoing (Nelson et al., 2006). CH may alter these processes in the hippocampus, a structure important in acquisition of long-term memory (Nelson, 1995) and vulnerable to injury from adverse conditions during the perinatal period (Rao et al., 2003; Towfighi et al., 1997).

Using *in vivo* ^{1}H NMR spectroscopy, we have previously demonstrated that CH has an adverse effect on the developing rat hippocampus. CH caused significant alterations in energy metabolism and glutamatergic neurotransmission, as suggested by increased phosphocreatine to creatine (PCr/Cr) ratio and altered glutamate to glutamine ratio (Raman et al., 2005). The increased PCr/Cr ratio in the presence of decreased oxidative phosphorylation that has been demonstrated in a similar animal model (LaManna et al., 1996; Caceda et al., 2001) suggests reduced energy utilization in the hippocampus, presumably as an adaptive response to CH. The altered glutamate–glutamine ratio in the CH hippocampus suggests that the decreased energy utilization may occur through suppression of glutamate-mediated excitatory neurotransmission, a metabolically expensive process (Attwell and Laughlin, 2001). While such adaptation may be beneficial in the short term, it may have deleterious effects in the long-term, since glutamate plays an important role in synaptogenesis through stimulation of NMDA receptors in the developing brain (Monnerie et al., 2003). Based on this, we hypothesized that CH would alter dendritic morphology in the hippocampus.

Dendritic growth is essential for synaptogenesis, and variations in dendritic morphology exert a critical influence on neuronal information processing (Hausser et al., 2000). Dendritic growth and development are highly dynamic processes. Peak neurite formation and extension within the hippocampus occur in the first postnatal month in rats (Pokorny and Yamamoto, 1981). After the establishment of the initial segment length, branching occurs, and as the animal matures, dendritic segment length decreases with increasing branching (Pokorny and Yamamoto, 1981).

The first objective of our study was to demonstrate acute and chronic alterations in the apical segment length of the dendrites in the hippocampus following CH using microtubule-associated protein-2 (MAP-2) histochemistry. We studied the apical segment length of dendrites on postnatal day (P) 28 (i.e., while rats were hypoxic) and on P56 after the rats had been normoxic for 4 weeks.

Expression of MAP-2 has been used to study dendritic morphology in the hippocampus (Jorgenson et al., 2003). MAP-2 is the most abundant MAP in the brain and is highly compartmentalized in the cell body and dendrites while being excluded from the axons (Bernhardt and Matus, 1984). MAP-2 is a highly energy-sensitive protein (Avila et al., 1994). There is an established correlation between increases in phosphorylation of MAP-2 and increases in dendritic branching (Diez-Guerra and Avila, 1993). Disruption of MAP-2 immunostaining following an insult to the brain is postulated to be the result of a cascade of biochemical events. This cascade results in calcium influx into the cell with activation of Ca2+/Calmodulin kinase II (CAMKII), which may result in abnormal phosphorylation and impaired dendritic structure and function (Fineman et al., 1993). Altered apical segment in the CA1 subarea has been demonstrated in perinatal iron deficiency, an energy-compromised condition (deUngria et al., 2000) similar to CH (Jorgenson et al., 2003).

Altered hippocampal structure and neurochemistry due to CH may be expected to affect hippocampus-dependent memory. Hence, the second objective was to assess long-term hippocampus-based cognitive impairments due to CH using auditory trace and delay Pavlovian fear conditioning on P56.

In Pavlovian fear conditioning, fear is acquired to a conditioned stimulus (CS), such as an auditory tone after it has been paired with an unconditioned stimulus (US), such as an electric shock (Gewirtz and Davis, 2000). In the delay fear conditioning paradigm, the CS and the US overlap in time, whereas in a trace fear conditioning paradigm, the US is delivered at a given time interval after the end of the CS. Trace, but not delay fear conditioning is susceptible to lesions of the dorsal hippocampus in rats (Gewirtz and Davis, 2000; Quinn et al., 2005).

2. Results

The body weight of the rats in the CH group was decreased by 60% on P28 and 49% on P56, when compared with the normoxic controls (Table 1). The brain weight was decreased by 56% on P28 and 17% on P56. The hematocrit was elevated in

Table 1 – Effect of chronic hypoxia on body weight, brain weight, hematocrit, and brain iron concentration of rats on postnatal days 28 and 56

	Age (days)	Chronic hypoxia	Normoxia	*p*-value
Body weight(g)	28	61.1±2.14	97.5±3.91	0.00
	56	226.0±1.34	337.5±5.06	0.00
Brain weight* (g)	28	0.73±0.01	1.14±0.01	0.00
	56	1.73±0.08	2.03±0.01	0.03
Hematocrit (%)	28	65.1±1.10	39.3±0.49	0.00
	56	50.75±1.59	44.5±1.24	0.05
Brain iron* (μg/g)	56	54.68±3.15	51.8±4.80	0.64

Values: mean±SEM (n=8/group at each age except *n=4).
Significance by two-tailed unpaired t test.