

NEUROPROTECTIVE ACTIONS OF TETRAHYDROBIOPTERIN AGAINST HYPOXIA- INDUCED MOTOR IMPAIRMENTS IN THE FETAL BRAIN

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BACKGROUND: Genetic deficiency in tetrahydrobiopterin (BH₄) is associated with dystonia and other developmental abnormalities. Our rabbit model of hypoxia-ischemia (HI) exhibits characteristic features of dystonia. HI at gestational day 22 (E22) results in >80% of death or motor deficits after delivery (E32). We have also shown that BH₄ and GTP cyclohydrolase levels in the E22 fetal rabbit brain are significantly lower than postnatal day 1 (E32), indicating that BH₄ pathway is developmentally regulated.

OBJECTIVE: We hypothesized that BH₄ is a critical factor for the normal development of the fetal brain. We investigated the developmental changes of BH₄ in different regions of the brain before and after HI and whether supplementation of BH₄ would ameliorate the motor deficits seen in the rabbit model.

METHODS: The BH₄ content in parts of the brain was analyzed by HPLC with electrochemical detection. The nNOS mRNA were determined by quantitative RT-PCR in perinatal rabbit brain at gestational ages 22 (E22), E25, E29 and E32 (postnatal day 1). Fetal HI was induced by subjecting the rabbit dams at E22 to 40 min uterine ischemia. For supplementation studies, sepiapterin, a precursor of BH₄, or vehicle was continuously delivered to dams using an Alzet mini pump (0.6 mg/kg/day) for a week prior to HI and behavior after delivery at E32 was assessed.

RESULTS: BH₄ is found in the highest levels in the thalamus compared to frontal, occipital, hippocampus, basal ganglia and parietal cortex. Thalamic BH₄ concentrations varied from 14.9±1.61 pmoles/mg protein at (E22) to 25.9±6.83 pmoles/mg protein (E29). Following HI the thalamus showed a significant decrease in BH₄ levels of ~25% followed by ~19% in basal ganglia levels. In contrast, ascorbate levels, which are significantly higher than BH₄, were decreased across all brain regions after HI. Maternal supplementation with sepiapterin doubled BH₄ levels from 14.9±1.6 to 30.56±4.16 pmoles/mg protein in the thalamus while only a 30% increase in basal ganglia was found. The nNOS gene expression also increase with age and in the thalamus, nNOS expression was highest at E22. Nitrite levels however were not significantly modified in the E22 thalamus or basal ganglia following HI, indicating that loss of NO is not a mechanism explaining HI injury. Sepiapterin treatment compared to vehicle decreased the frequency of severe motor deficits or death from 80% to 37%, and increased the frequency of normal appearing kits following HI.

CONCLUSIONS: We conclude that BH₄ is an important neuroprotective factor for development of motor functions. Loss of thalamus BH₄ content critically correlates with HI damage to fetal brain. These findings suggest that BH₄ is a promising therapeutic agent for the prevention of fetal brain damage in a pregnant population at risk for HI.