

Creatine administration during pregnancy decreases expression of apoptotic markers in the newborn spiny mouse brain following birth hypoxia: further evidence for the neuroprotective effects of prophylactic creatine

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Objectives: We have recently shown in the precocial spiny mouse that supplementation of the maternal diet with 5% creatine monohydrate from mid-pregnancy significantly reduced mortality and improved postnatal growth of offspring following acute hypoxia at the time of birth (Ireland et al., *AJOG*, 2008;198). The mitochondrial protein Bax (a pro-apoptotic member of the BCL-2 family) and its effector enzyme Caspase-3 have been implicated in DNA injury in the hypoxic brain. As phosphorylated creatine (PCr) acts as an energy buffer in times of high energy demand, we hypothesised that increased fetal brain reserves of PCr may protect the brain against hypoxia-related apoptosis through maintenance of mitochondrial function, thereby stabilising membrane potentials and reducing the likelihood of metabolic acidosis and glutamate excitotoxicity.

Methods: Pregnant spiny mice were fed a control or 5% creatine-supplemented diet from day 20 of gestation (term ~39 days). On day 37-38, pups were delivered by caesarean section, or intrauterine hypoxia was induced by placing the excised uterus containing all fetuses in a saline bath for 7.5-8 mins, after which the fetuses were expelled and resuscitation attempted by manual palpation of the chest. Surviving neonates were cross-fostered to a nursing dam for 24 h. At post mortem fetal brains were immersion fixed and processed for Bax and caspase-3 immunohistochemistry.

Results: Bax immunoreactivity (IR) was higher in the amygdala, hypothalamus and piriform cortex of pups exposed to birth hypoxia, compared to both creatine + hypoxia pups, and control (caesarean section) pups. Low levels of Bax-IR (<150cells/field of view) was present in control brains, whereas intense Bax-IR (>400cells/field of view) was seen in all brain regions after birth hypoxia. In pups from the creatine + hypoxic group, moderate Bax-IR (<200cells/field of view) were observed in all brain regions examined. As determined by densitometric analysis, hypoxia pups showed greater Caspase-3-IR in CA3 (p<.05) and CA1 (p=0.07) regions of the hippocampus in comparison to their caesarean delivered controls. No such difference was seen between controls and creatine + hypoxia pups.

Conclusions: In the neonatal spiny mouse brain, intrauterine hypoxia resulted in increased Bax and Caspase-3-IR 24 h after insult. Region-specific increases were prevented with prophylactic maternal creatine supplementation. Mitochondrial preservation via creatine loading may play a central role in promoting cellular survival following intrauterine hypoxia. Investigation into these pathways is the focus of ongoing experiments.