Apocynin Decreases Consequences of Nitric Oxide Synthase Uncoupling in Experimental Neonatal Hypoxia-Ischemia

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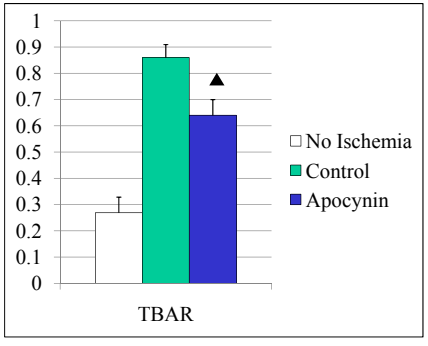
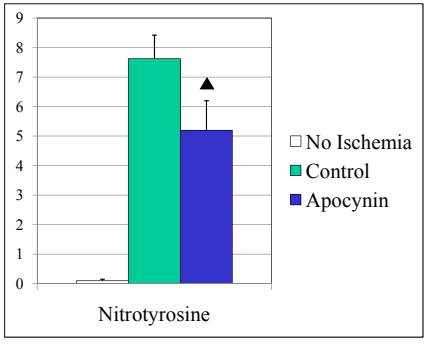
Purpose: To determine whether inhibiton of NADPH oxidase (NOX) could ameliorate oxidative brain injury following cerebral hypoxic/ischemic (HI) injury in the neonatal rat.

BACKGROUND: Oxidative injury accompanies HI injury. Since very little recruitment of neutrophils occurs in neonatal HI (1), we sought an alternative source for oxy-radicals and established uncoupling of nitric oxide synthase (NOS) as a potential mechanism (2). NOS uncoupling results from a dysfunctional enzyme in which superoxide radical is generated in lieu of nitric oxide. Pharmacological inhibition of NOX with apocynin ameliorated uncoupling, potentially through sparing of oxidative depletion of co-factors. NOX inhibition decreases cerebral injury from ischemia (3). Here we investigate whether NOX inhibition also ameliorates oxidative brain injury.

DESIGN/METHODS: Seven day old neonatal rats (P7 rats) were subjected to HI by ligating one common carotid artery under anesthesia and exposure to 8% oxygen for 90 minutes followed by resuscitation with 100% oxygen for 2 hours. Pups were then given an intraperitoneal injection of a carrier solution or apocynin, 25 mg/Kg. After a survival of 24 hours, pups were euthanized, and cortical tissue from both sides of the brain was homogenized. Samples were assayed for lipid oxidation products using a TBARS assay and for nitrotyrosine using an ELISA assay.



▲ - p = 0.035



▲ - p = 0.022

RESULTS: TBARS reactive products and nitrotyrosine were increased (120% and 500% respectively) in cerebral hypoxic-ischemic cerebral tissue. There was a significant decrease in TBARS reactive products (54%; p = 0.035, n = 5) and nitrotyrosine (32%; p = 0.022, n = 5) in hypoxic-ischemic tissue of pups treated with apocynin.

CONCLUSIONS/RELEVANCE: Inhibition of NOX following H/I injury was able to ameliorate oxidative damage to lipids and proteins, suggesting that NOX activation participates in the oxy-radical cascade that accompanies this condition. Further studies are in progress to determine the effect of this treatment on structural and functional outcome measures.

References:

1. J Neurosci Res 2007;85:1420-6.
2. Am J Physiol 2008;295:H1809-14.
3. J Int Med Res 2007;35:517-22.

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