ANTISENSE EVIDENCE FOR NF- B-MEDIATED SIGNAL TRANSDUCTION IN THE MECHANISM OF PHENYTOIN EMBRYOPATHIES.

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1. Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada; 2. Department of Pharmacology, University of Toronto, Toronto, ON, Canada The nuclear transcription factor-kappa B (NF-κB) family regulates expression of many genes, some of which have developmental roles. Excessive NF-κB activation is implicated in the pathogenesis of several diseases including cancer. NF-κB is activated by numerous stimuli, many of which increase the levels of reactive oxygen species (ROS), and activation is inhibited by some antioxidants. NF-κ B may also be a downstream effector of ROS-mediated signal transduction, such as the Ras pathway. Teratogens like the anticonvulsant drug phenytoin enhance embryonic ROS formation, and embryopathies may result from oxidative damage to cellular macromolecules, and/or enhanced ROS-mediated signaling. We previously showed that phenytoin increases embryonic Ras activation, and Ras inhibition blocks phenytoin embryotoxicity. To determine the toxicological role of ROS-mediated Ras activation of NF-κB signaling, embryos were cultured with either a therapeutic concentration of phenytoin (20 g/ml, 80 M) or its vehicle (0 002N NaOH), with or without antisense NF-κB (p65) (2 5 - 25 M). Gestational day 9 5 CD-1 embryos incubated with phenytoin showed decreases in anterior neuropore closure, turning, yolk sac diameter, crown rump length and somite development ($p<0.05$). Addition of antisense NF-κB to the culture medium decreased the embryotoxic effects of phenytoin on anterior neuropore closure, turning and somite development ($p<0.05$), but did not protect against decreases in yolk sac diameter and crown rump length. The protective effects were not observed using sense or nonsense controls, or antisense vehicle, suggesting that NF-κB-mediated signal transduction may play a role in the mechanism of phenytoin embryopathies.