

**Title:** A role for FLIP ([Fas associated death domain like interleukin-1 $\beta$ -converting enzyme] - inhibitory protein) in neuroprotection induced by hypoxic preconditioning

**Authors:** KS Payton, RA Sheldon, DM Ferriero and FJ Northington

**Affiliation:** Neonatal Research Laboratory, Dept of Pediatrics, Johns Hopkins University School of Medicine; Neonatal Brain Disorders Laboratory, UCSF School of Medicine

**Background:** Signaling through the Fas death receptor (Fas DR) pathway contributes to neurodegeneration following neonatal hypoxia-ischemia (HI). Hypoxic preconditioning provides protection against future HI injury, but the mechanisms underlying this protection are not fully understood. We have shown that levels of Flip [Fas –associated death domain-like interleukin-1 $\beta$  converting enzyme]-inhibitory protein, a dominant negative inhibitor of caspase 8, are altered by antioxidant status and are modulated by exposure to hypoxia. The ratio of Flip protein expression to Fas DR expression is highly predictive of degree of injury following neonatal HI.

**Objective:** To determine the effect of hypoxic preconditioning on levels of Fas DR and on levels of both isoforms of Flip- Flip Long (Flip<sub>L</sub>) and Flip Short (Flip<sub>S</sub>) following HI.

**Methods:** Six day old wild type mice were exposed to FiO<sub>2</sub>=0.08 or room air x 30 minutes and then 24 hours later exposed to HI (ligation of R common carotid and FiO<sub>2</sub>=0.08 x 45 minutes). Animals were killed at 2 hours and 24 hours following HI. Crude homogenates of ipsilateral cortex were used to determine expression of Fas death receptor protein and both isoforms of Flip. Preliminary immunoprecipitation experiments with FADD were performed to determine if Flip is functionally bound to the death inducing signaling complex (DISC) and if there were differences in Flip binding at 2 hours following HI in preconditioned and non-preconditioned animals.

**Results:** Levels of total Flip, and Flip<sub>L</sub> were less at 2 hours in preconditioned HI cortex (total Flip 0.439 $\pm$ .273, Flip<sub>L</sub> 0.081 $\pm$ 0.059, Flip<sub>S</sub> 0.358 $\pm$ 0.220) relative to non-preconditioned HI cortex (total Flip 0.97 $\pm$ .72, Flip<sub>L</sub> 0.288 $\pm$ .144, Flip<sub>S</sub> 0.682 $\pm$ .59, p=0.05). Levels of Flip<sub>L</sub> and Flip<sub>S</sub> increased 2.7 and 2.3 fold respectively, between 2 and 24 hours in preconditioned HI cortex, but was unchanged between 2 and 24 hours in non-preconditioned HI cortex. There was no difference in levels of Fas DR between the two groups at either 2 or 24 hours after HI. Preliminary immuno-precipitation studies show that both Flip<sub>L</sub> and Flip<sub>S</sub> are strongly bound to the DISC in sham samples and that there is less Flip associated with the DISC at 2 hours in preconditioned HI cortex compared to non-preconditioned cortex.

**Conclusions:** The main effects of preconditioning are to decrease the initial (2h) levels of Flip and to subsequently enhance the expression of Flip between 2 and 24 hours after HI compared to non-preconditioned HI cortex. Fas DR is unaffected by hypoxic preconditioning. Functional binding of Flip at 2 hours after HI reflects the relative levels of Flip expression. If a portion of the neuroprotection provided by hypoxic preconditioning is mediated through the Fas death receptor pathway, it is likely due to the ability of preconditioned animals to increase Flip levels between 2 and 24 hours in the ipsilateral cortex following neonatal HI.