

Activated Src kinases interact with the NMDA receptor after neonatal brain ischemia

Xiangning Jiang,¹ Dezhi Mu,^{1,2} Valerie Biran,³ Joel Faustino,¹ Shengjun Chang,¹
Christina M. Rincón,⁴ R. Ann Sheldon,¹ and Donna M. Ferriero^{1,5}

Departments of ¹Neurology, ⁵Pediatrics, University of California San Francisco, CA, 94143

²Department of Pediatrics, West China Second University Hospital, Sichuan University,
Chengdu, Sichuan 610041, China

³Department of Neonatology, Hôpital Trousseau, AP-HP, University of Paris VI, Paris, France

⁴Brown University Medical School, 69 Brown Street, Box G-8089, Providence, RI 02912

Background and Objective: Neonatal stroke is associated with the N-Methyl-D-Aspartate receptor (NMDAR)-mediated excitotoxic brain injury. Src family kinases (SFKs) are considered to be the molecular hub for NMDAR regulation. We determined the relationship between SFKs activation and NMDAR tyrosine phosphorylation following neonatal hypoxia-ischemia (HI) and investigated the neuroprotective potential of a selective SFKs inhibitor, PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo [3, 4-d] pyrimidine), against neonatal brain ischemic injury

Methods: The Rice-Vannucci model was adapted for neonatal HI injury in postnatal day 7 CD1 mice. SFKs activity in the postsynaptic densities (PSD) was measured by Western blotting. NMDAR tyrosine phosphorylation and their association with SFKs were determined by co-immunoprecipitation. Brains from animals treated with PP2 at 30min after HI (1µg/g, i.p.) or its inactive analog, PP3 (1 µg/g, i.p.), were examined histologically 5 days after HI procedure with cresyl violet and iron stain to assess the degree of damage.

Results: Neonatal HI resulted in a rapid and transient increase in tyrosine phosphorylation of NMDAR subunits NR2A (at 0min, 15min and 1hr of reperfusion) and NR2B (at 0min and 15min of reperfusion). This up-regulation correlated with the enhanced association of Fyn and Src with NR2A and NR2B (both increased from 0min to 6hr after HI). SFKs were activated in the PSD following neonatal HI (from 15min through 6hr of reperfusion). PP2 treatment (n = 11, median score = 15) attenuated brain injury when compared to saline vehicle controls (n = 12, median score = 20, p = 0.0017 vs PP2 group) following neonatal HI, whereas PP3 (n = 9, median score = 21, p = 0.007 vs PP2 group) did not protect the brain from the HI insult.

Conclusions: SFKs may play an important role in NMDAR-mediated excitotoxicity and downstream events leading to neuronal death after neonatal HI. Inhibition of SFKs may provide protection against neonatal stroke. Rather than blockade of NMDAR after HI in the developing brain, it may be safer and more beneficial to manipulate components of the NMDAR signaling complex at the PSD.

This work was supported by American Heart Association grant 0430173N, University of California San Francisco REAC grant to Xiangning Jiang, NIH 3RO1 NS 33997 to Donna M. Ferriero and National Natural Science Foundation of China 30570623, 30770748 to Dezhi Mu.