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Function of prostaglandin E_2 EP receptors in the acute outcome of rodent hypoxic ischemic encephalopathy

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ABSTRACT

Neonatal hypoxic-ischemic encephalopathy (HIE) is a leading cause of severe and permanent neurologic disability after birth. The inducible cyclooxygenase COX-2, which along with COX-1 catalyzes the first committed step in prostaglandin (PG) synthesis, elicits significant brain injury in models of cerebral ischemia; however its downstream PG receptor pathways trigger both toxic and paradoxically protective effects. Here, we investigated the function of PGE_2 E-prostanoid (EP) receptors in the acute outcome of hypoxic-ischemic (HI) injury in the neonatal rat. We determined the temporal and cellular expression patterns of the EP1–4 receptors before and after HIE and tested whether modulation of EP1–4 receptor function could protect against cerebral injury acutely after HIE. All four EP receptors were expressed in forebrain neurons and were induced in endothelial cells after HIE. Inhibition of EP1 signaling with the selective antagonist SC-51089 or co-activation of EP2–4 receptors with the agonist misoprostol significantly reduced HIE cerebral injury 24 h after injury. These receptor ligands also protected brain endothelial cells subjected to oxygen glucose deprivation, suggesting that activation of EP receptor signaling is directly cytoprotective. These data indicate that the G-protein coupled EP receptors may be amenable to pharmacologic targeting in the acute setting of neonatal HIE.

Hypoxic-ischemic encephalopathy (HIE) in the neonate is one of the leading causes of cerebral palsy, mental retardation, and learning disabilities, making it critical to identify therapeutic strategies to reduce brain injury in HIE. The inducible cyclooxygenase COX-2 has been implicated in cerebral injury in models of adult stroke [8], and is induced in HIE where it is associated with acute hypoxic-ischemic events [18].

The cyclooxygenases COX-1 and COX-2 catalyze the first committed step in the formation of prostaglandins from arachidonic acid. COX-2 is rapidly upregulated in neurons following *N*-methyl-D-aspartate (NMDA) receptor-dependent synaptic activity [26], consistent with a physiologic role in modulating synaptic plasticity. COX-2 expression and activity are also induced in neurons in vivo in acute paradigms of excitotoxicity, and promote ischemic injury (reviewed in [8]). In models of neurodegeneration, where inflammation is a dominant pathological component, COX-2 is upregulated in microglia, where it is promotes secondary neuronal injury [8]. The mechanism by which COX-2 activity promotes neurotoxicity is due to actions of one or more downstream prostaglandin (PG) signaling pathways through specific PG receptors. The five downstream prostanoid products of COX activity, PGE_2, PGF_{2a}, PGD_2, PGI_2 (prostacyclin), and TxA_2 (thromboxane A_2) bind to specific G-protein coupled receptors designated EP (for E-prostanoid), FP, DP, IP, and TP receptors, respectively [5]. PG receptor subtypes are distinguished by which PG they bind, and by the signal transduction pathway that is activated upon ligand binding. Activation of PG receptors leads to changes in the production of cAMP and/or phosphoinositol turnover and intracellular Ca^{2+} mobilization. Further complexity occurs in the case of PGE_2, which can bind four receptor subtypes (EP1, EP2, EP3, and EP4) with distinct and potentially antagonistic signaling cascades.

PGE_2 is a major downstream product of COX-2 enzymatic activity and a potent lipid messenger that activates four distinct G-protein coupled receptors, the EP1–EP4 receptors. In experimental stroke, levels of COX-2 and PGE_2 are markedly upregulated [15] and COX-2 can exert neurotoxicity via the Ga_q-coupled EP1 receptor in vivo [2,9,27]. However, neuronal PG receptors that are positively coupled to cAMP can elicit paradoxical protective effects in vitro in excitotoxic and hypoxic paradigms [4,12,14], indicating that both protective as well as toxic PG signaling pathways may be

Abbreviations: COX, cyclooxygenase; EP, E-prostanoid; HI, hypoxia-ischemia; HIE, hypoxic ischemic encephalopathy; NSAID, non-steroidal anti-inflammatory drug; OGD, oxygen-glucose deprivation; PFA, paraformaldehyde; PG, prostaglandin; PI, propidium iodine; TTC, 2,3,5-triphenyl-tetrazolium chloride.

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active in cerebral ischemia [3,13,14]. In this study, we examined the function of the PGE_2 EP receptors in acute outcome in a rodent model of HIE using pharmacologic strategies. We identified cerebroprotective effects of misoprostol, an agonist to the EP2–4 receptors as well as the EP1 antagonist SC-51089.

All animal experiments were approved by Administrative Panel on Laboratory Animal Care (APLAC) at Stanford University. The rodent model of neonatal rat HIE was carried out according to the protocol established by Rice et al. and as previously described [17,21]. Sham treated animals underwent ligation of the carotid artery but no hypoxia. Vehicle, SC-51089 (10 μg/kg), misoprostol (50 μg/kg or 500 μg/kg) were administered intraperitoneally either 5 min before or 5 min after hypoxia. Twenty-four hours after HI, Sprague Dawley rat pups were lethally anesthetized and brain tissue was harvested for infarct quantification using 1% 2,3,5-triphenyl-tetrazolium chloride (TTC) staining in a blinded fashion [21].

Rat pups (n = 2–3 per time point) were deeply anesthetized with isoflurane and transcardially perfused with normal saline followed by 4% PFA, then overnight fixation in PFA at 4 °C. Brains were processed for paraffin sections (5 μm thickness). Infarct area was determined with cresyl violet staining. Following antigen retrieval (in citrate pH 6.0, boiled in a microwave for 20 min) and permeabilization with 0.3% triton together with blocking with 10% normal goat serum for 1 h, immunostaining for EP1–4 was carried out using anti-EP1–4 polyclonal antibodies (1/500; Cayman Chemical,

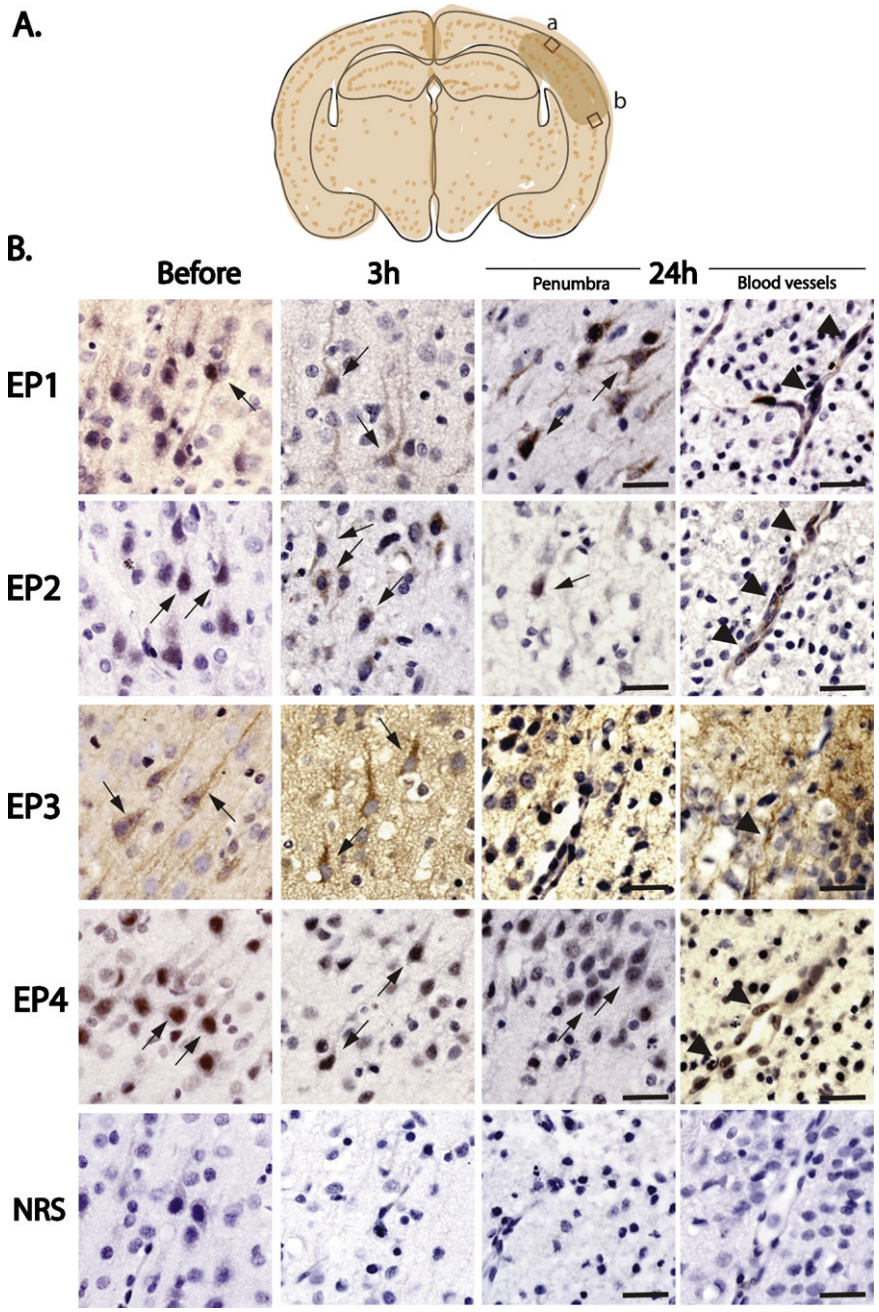

Fig. 1. Immunohistochemistry of EP1–4 receptors in postnatal rat HIE brains. (A) Schema of the cortical areas examined (box a: within infarct zone; box b: penumbra). EP1–4 receptor expression was examined in cerebral cortex in layers II/III dorsally and ventrally to the infarct area in the penumbra at 3 h and 24 h after HI. (B) EP1–4 receptors are dynamically regulated in sham (just before onset of hypoxia) and HI (3 h and 24 h after hypoxia) pups in cerebral cortical neurons (thin arrows) and microvasculature (arrowheads). Normal rabbit serum (NRS) control stains did not show specific staining. Scale bar = 50 μm.