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COMMENTARY

Prostaglandin signalling in cerebral ischaemia

Katrin Andreasson

Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, USA

The inducible cyclooxygenase COX-2 exerts neurotoxic effects in a wide spectrum of neurological disease models, including models of cerebral ischaemia and chronic neurodegeneration. As COX-1 and COX-2 catalyse the first committed step in prostaglandin synthesis, recent efforts have focused on identifying the downstream prostaglandin signalling pathways responsible for mediating the toxic effect of COX-2. Recent studies in models of *in vitro* excitotoxicity or hypoxia demonstrate that certain prostaglandin receptors mediate toxic effects, but a large number appear to mediate paradoxically protective effects. *In vivo* studies have begun to confirm initial *in vitro* findings, with selected prostaglandin receptors eliciting either neurotoxic or protective effects in models of cerebral ischaemia. In the present issue, Ikeda-Matsuo *et al.* examine the function of the PGE_2 EP3 receptor in a model of transient focal ischaemia and explore its potential signalling through Rho kinase activation.
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Keywords: COX-2; PGE_2; EP1 receptor; EP2 receptor; EP3 receptor; EP4 receptor; excitotoxicity; cerebral ischaemia; vasculature

Abbreviations: COX-2, cyclooxygenase-2; EP, E-prostanoid; mPGES, microsomal prostaglandin E2 synthase; NVU, neurovascular unit; PG, prostaglandin

COX-2 is the inducible form of cyclooxygenase, and catalyses the first committed step in the formation of prostaglandins (PGs) from arachidonic acid. In brain, COX-2 expression and PG production increase markedly in neurons, glial cells and endothelial cells following a variety of stimuli including excitatory synaptic activity, growth factors, hypoxia and inflammatory mediators. Previous studies in rodent stroke models have shown that COX-2 enzymatic activity leads to neuronal death and increased stroke injury, and inhibition of COX-2-dependent PG generation using pharmacological or genetical strategies reduces infarct volume (reviewed in Hewett *et al.*, 2006). Thus, an important effort is now underway to examine the downstream PG receptor pathways responsible for COX-2-mediated cerebral injury in stroke.

However, studying the specific effects of PG signalling is complicated, not only because there are five prostanoids [PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 (prostacyclin) and thromboxane A_2], and nine distinct PG receptors [E-prostanoid (EP1–4), FP, DP1–2, IP and TP respectively], but because activation of PG receptors elicits specific changes in production of cAMP, phosphoinositol turnover, and/or intracellular Ca^{2+} mobilization, depending on whether the receptor is coupled to G_s, G_i, G_q or $G_{12/13}$. Moreover, PG receptors in brain are expressed in multiple cell types, have different cellular expression profiles and differ in ligand binding and desensitization kinetics.

In the face of this daunting complexity, pharmacological and genetical approaches have begun to make significant inroads into understanding the function of COX-2/PG signalling in cerebral ischaemia. *In vitro* modelling to test the function of individual PG receptors in paradigms of excitotoxicity and hypoxia has revealed that several PG receptors do indeed mediate neurotoxicity; however, other PG receptors are paradoxically protective. For the PGE_2 EP1–4 receptors in particular (reviewed in Andreasson, 2009), the EP1 receptor mediates a significant component of COX-2 toxicity, as demonstrated by Kawano *et al.* (2006) in elegant combinations of genetics and pharmacology (see also Ahmad *et al.*, 2006). In contrast, the PGE_2 EP2 receptor mediates paradoxical cerebroprotection in models of cerebral ischaemia (McCullough *et al.*, 2004; Liu *et al.*, 2005). Misoprostol, an anti-ulcer therapeutic agent that binds to the EP2–4 receptors exerts significant cerebroprotective effects via the EP2 and/or EP4 receptors (Li *et al.*, 2008). In the current study, Ikeda-Matsuo *et al.* (2010) investigate the role of the EP3 receptor, which is the most complex of the four EP receptors in that it is differentially spliced to form several isoforms that differ in their carboxy terminus and therefore in signalling properties. In this regard, the authors focus specifically on the coupling of the EP3 receptor to $G_{12/13}$, one of several signalling pathways for EP3, which results in activation of Rho kinase.

Correspondence: Katrin Andreasson, Stanford University School of Medicine, Department of Neurology and Neurological Sciences, 1201 Welch Road, MSLS P210, Stanford, CA 94305, USA. E-mail: kandreas@stanford.edu
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In initial *in vitro* experiments, the authors found that the EP3 antagonist ONO-AE3-240 rescued hippocampal CA1 neuronal death in glutamate-treated rat organotypic hippocampal slices, and stimulation with the EP3 agonist ONO-AE1-248 enhanced injury in microsomal prostaglandin E2 synthase (mPGES) null mouse slices. One caveat in interpreting these data centres on the fact that activation of the EP1 receptor, which induces hippocampal CA1 neuronal death in this model (Kawano *et al*., 2006; Zhou *et al*., 2008), did not result in neurotoxicity; moreover, administration of sulprostone, an EP3 agonist at low nanomolar concentrations, has been previously shown to be neuroprotective in organoptyic hippocampal slices (Wu *et al*., 2007) and in spinal cord slices where it is associated with an increase in the pro-survival phospho-Akt (Bilak *et al*., 2004). Perhaps this difference reflects a technical issue in the way the CA1 propidium iodide (PI) fluorescence was quantified in the present study: in the EP1 and sulprostone studies, hippocampal PI fluorescence was quantified using a strategy to normalize each hippocampal slice to itself to control for potential differences in slice size and glutamate receptor levels that vary as one progresses from rostral to caudal hippocampus. *In vivo*, the authors show that administration of EP3 antagonist as well as genetic deletion of the inducible mPGES significantly decreased infarct size in the 2 h transient focal ischaemia/reperfusion model. Although the authors assessed the effect of adding an EP3 antagonist to the mPGES knockout stroke model, the converse addition of an EP3 agonist to mPGES–/– mice could help assess whether restoring EP3 signalling in a PGE_2 depleted state increases cerebral injury. The authors conclude their study by examining the function of EP3-stimulated Rho kinase activity in hippocampal slices, where they note an EP3-mediated increase in phosphorylation of myelin-binding subunit, a substrate of Rho kinase, under basal conditions. Future studies should hopefully explore the intriguing hypothesis that EP3 signalling is toxic *in vivo* via a $G_{12/13}$-Rho kinase pathway.

An emerging concept in cerebrovascular research is the importance of the neurovascular unit (NVU), which is comprised of endothelial cells, astrocytes, pericytes and smooth muscle cells, along with neuronal inputs and perivascular microglia. The NVU participates in physiological coupling of neuronal activity and cerebral blood flow that is dependent on COX-2 activity and PG signalling (Niwa *et al*., 2000). In addition, EP receptors, at least in the systemic circulation, are known to modulate vascular tone through vasodilation (EP2, EP4) or vasoconstriction (EP1, EP3). Given the role of EP receptors in vascular tone, these receptors may modulate cerebral blood flow dynamics in models of cerebral ischaemia. Thus, one of the major challenges in understanding the mechanisms of EP receptor signalling in brain, and in particular in cerebral ischaemia, is defining the specific cellular PG signalling pathways in the NVU that mediate their toxic or protective effects *in vivo*. For example, toxic EP1 signalling in cerebral ischaemia may be mediated by neuronal EP1 signalling, which *in vitro* can alter levels of pro-survival phospho-Akt (Zhou *et al*., 2008) and in addition can affect Na^+/Ca^{2+} exchanger function (Kawano *et al*., 2006). However, vascular EP1 may be important as well, as deletion of the EP1 receptor appears to improve cerebral blood flow, potentially reducing the severity of cerebral ischaemia (Saleem *et al*., 2007). The EP2 receptor, which functions protectively in cerebral ischaemia and promotes neuronal survival via PKA-dependent neuronal signalling (McCullough *et al*., 2004), is also known to have vasodilatory functions. EP3, in addition to signalling in neurons and vasculature, is also known to affect platelet aggregation and perhaps microglial inflammatory functions as well. Finally, in a broader context, EP receptors may function very differently depending on the injury paradigm. For example, the EP2 receptor, which is cerebroprotective in models of cerebral ischaemia, functions in a pro-inflammatory and neurotoxic manner in models of innate immunity, Familial Alzheimer's disease, and amyotrophic lateral sclerosis (Montine *et al*., 2002; Liang *et al*., 2005; 2008). This dichotomy of action may reflect different cell-specific EP2 signalling cascades, for example neuronal and/or vascular cerebroprotective EP2 signalling in stroke models, as opposed to pro-inflammatory microglial EP2 signalling in chronic inflammatory neurodegeneration. The broad repertoire of cellular EP signalling, and of PG receptors more generally, underscores one of the major challenges in dissecting out the function of PG receptor signalling in brain. However, this effort is worthwhile, in part because of the dramatic effects that individual PG receptors exert in models of cerebral ischaemia, and because PG receptors are G protein-coupled receptors, a class of receptors that historically has been successfully targeted therapeutically.

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