

## Cyclooxygenase-2 in epilepsy

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### SUMMARY



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Epilepsy is one of the more prevalent neurologic disorders in the world, affecting approximately 50 million people of different ages and backgrounds. Epileptic seizures propagating through both lobes of the forebrain can have permanent debilitating effects on a patient's cognitive and somatosensory brain functions. Epilepsy, defined by the sporadic occurrence of spontaneous recurrent seizures (SRS), is often accompanied by inflammation of the brain. Pronounced increases in the expression of key inflammatory mediators (e.g., interleukin  $-1\beta$  [IL- $1\beta$ ], tumor necrosis factor alpha [TNF $\alpha$ ], cyclooxygenase-2 [COX-2], and C-X-C motif chemokine 10 [CXCL10]) after seizures may cause secondary damage in the brain and increase the likelihood of repetitive seizures. The COX-2 enzyme is induced rapidly during seizures. The increased level of COX-2 in specific areas of the epileptic brain can help to identify regions of seizure-induced brain inflammation. A good deal of effort has been expended to determine whether COX-2 inhibition might be neuroprotective and represent an adjunct therapeutic strategy along with antiepileptic drugs used to treat epilepsy. However, the effectiveness of COX-2 inhibitors on epilepsy animal models appears to depend on the timing of administration. With all of the effort placed on making use of COX-2 inhibitors as therapeutic agents for the treatment of epilepsy, inflammation, and neurodegenerative diseases there has yet to be a selective and potent COX-2 inhibitor that has shown a clear therapeutic outcome with acceptable side effects.

**KEY WORDS:** Seizure, Neurodegeneration, Anticonvulsant, Prostaglandin, Blood-brain barrier, Cognitive deficit, EP2, EP1.

Injury or seizures can trigger multidimensional local inflammatory reactions in the brain, primarily involving activated microglia and infiltrating monocytes but also reactive astrocytes and neurons. The roles of cyclooxygenase-2 (PTGS2, or COX-2) in the consequences of seizures, and in epilepsy, have received much attention since the finding that seizures induce COX-2 in hippocampal principal neurons within hours (Yamagata et al., 1993; Marcheselli & Bazan, 1996), partly via a pathway involving *N*-methyl-D-aspartate (NMDA) receptors (Yamagata et al., 1993). COX-2 is also induced within days of a seizure in astrocytes

(Hirst et al., 1999), although nonaldehyde fixation is apparently needed to reveal astrocytic COX-2.

Cyclooxygenases 1 and 2 are membrane-associated proteins that catalyze the conversion of arachidonic acid to prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) in two steps mediated by separate enzymatic activities. First, the cyclooxygenase activity itself converts arachidonic acid to prostaglandin G<sub>2</sub> (PGG<sub>2</sub>), and then a peroxidase activity reduces PGG<sub>2</sub> to PGH<sub>2</sub>. PGH<sub>2</sub> in turn is rapidly converted by specific synthases to one of five prostanoids: thromboxane A<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , PGE<sub>2</sub>, prostacyclin (PGI<sub>2</sub>), or PGD<sub>2</sub> (Fig. 1). Four G-protein-coupled receptors are activated by PGE<sub>2</sub>, and two by PGD<sub>2</sub>, whereas each of the other three prostanoids activates a single receptor. The thromboxane receptor TP and the EP3 receptor have multiple splice variants that differ in their C-terminal tails (Abramovitz et al., 2000), but the functions of these isoforms have not been uncovered. COX-2 is constitutively expressed at low to moderate levels in both cell

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bodies and dendritic spines of excitatory hippocampal neurons, and it is regulated strongly by synaptic activity (Kaufmann et al., 1996). By contrast, perhaps all or at least some inhibitory interneurons in the hippocampus, such as those expressing somatostatin, do not express COX-2 (Serrano et al., 2011). Each COX-2 molecule undergoes suicide inactivation after converting about 400 arachidonate substrate molecules (Smith et al., 1996), which allow COX-2 to respond quite dynamically to fluctuating levels of neuronal activity. Cytosolic prostaglandin E synthase and microsomal prostaglandin E synthase-2 are constitutively expressed, whereas microsomal prostaglandin E synthase-1 is inducible and is often coupled to COX-2 (Murakami et al., 2000), such that PGE<sub>2</sub> is a prominent product of induced COX-2. Most cells predominantly express a single prostanoid synthase and therefore only a single principal prostanoid (FitzGerald, 2003), which aids analysis of a complex tissue response if the synthases can be localized. For example, although COX-2 is generally regarded as a proinflammatory enzyme, Gilroy et al. (1999) showed that COX-2-derived PGE<sub>2</sub> released from infiltrating polymorphonuclear leukocytes is proinflammatory in the acute phase of carrageenin-induced pleurisy, whereas PGD<sub>2</sub> released from infiltrating monocytes exerts an antiinflammatory role during the resolution phase. Antiinflammatory actions of nonneuronal COX-2 in the brain have been suggested by studies involving direct activation of the glial innate immune system by bacterial lipopolysaccharide (Aid & Bosetti, 2011), which hinders the emergence of a simple picture of COX-2 function in epilepsy. Indeed, activation of a single PGE<sub>2</sub> receptor (EP2) has recently been shown to exacerbate the rapid upregulation of interleukin-6 (IL-6) and IL-1 $\beta$  in classically activated microglia but to blunt the production of tumor necrosis factor alpha (TNF- $\alpha$ ), IL-10, Chemokine (C-C motif) ligand 3 (CCL3), and Chemokine (C-C motif) ligand 4 (CCL4) (Quan et al., 2013). EP2 thus regulates innate immunity in the central nervous system in a nuanced manner by promoting many aspects of inflammation while dampening others.

COX-2 involvement in seizures has been investigated intensely in both *in vitro* and *in vivo* models of neuronal hyperexcitability and excitotoxicity by making use of two key tools: genetically manipulated mice that lack or overexpress COX-2 either globally or conditionally, and COX-2 inhibitors (selective and nonselective). The premise for these studies is that loss of COX-2 function may prove beneficial in reducing acute seizure severity, intensity, and frequency. This review addresses a number of topics related to the roles of COX-2 and its prostanoid products in epilepsy, including (1) setting the seizure threshold both acutely and in chronic epilepsy, (2) regulating the integrity of the blood-brain barrier and the p-glycoprotein transporter after seizures, and (3) causing neuronal cell loss and inflammation following seizures.

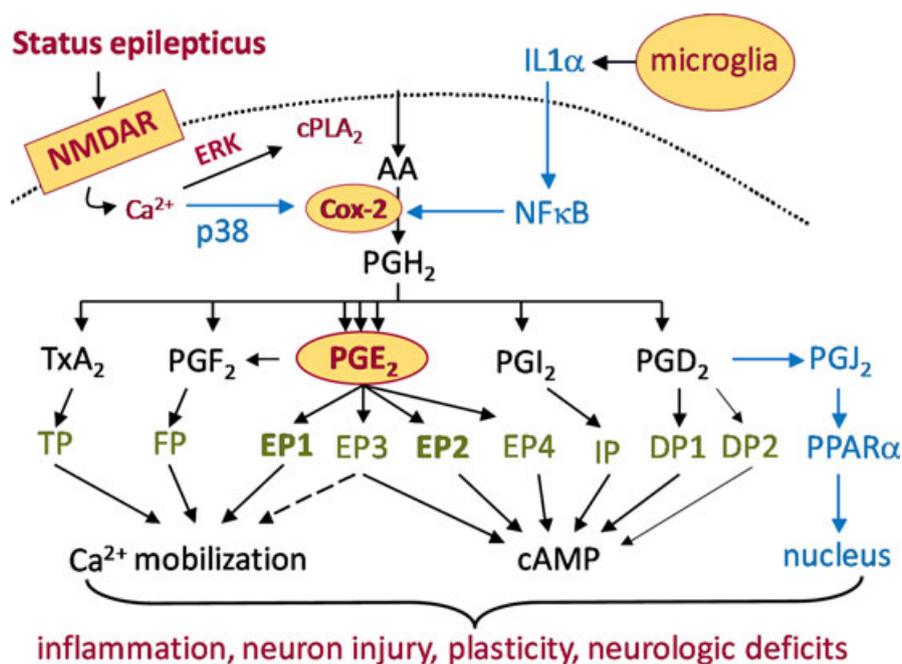
## COX-2 INHIBITORS: BASIS FOR SELECTIVITY

The structural basis for selectivity of the COX inhibitors has been revealed by x-ray and molecular modeling studies (Kurumbail et al., 1996; Luong et al., 1996; Filizola et al., 1997). These studies showed that COX-1 and COX-2 isozymes share 60–65% sequence identity and a conserved overall structure including the substrate binding site and catalytic region. However, subtle differences at the substrate binding site lead to inhibitor selectivity. Both isozymes contain three distinct domains, an N-terminal epidermal growth factor (EGF) domain followed by a membrane interaction motif and a C-terminal catalytic domain that harbors the cyclooxygenase and peroxidase catalytic regions. The cyclooxygenase pocket is composed of a long hydrophobic channel that extends from the membrane binding domain to a nearby heme group, which is oxidized to initiate the cyclooxygenation reaction. COX-2 contains a valine at amino acid positions 434 and 523, whereas COX-1 has isoleucine at the corresponding positions. The difference in the nature of these amino acids produces a more flexible binding pocket in COX-2 compared to COX-1 (Kurumbail et al., 1996; Luong et al., 1996). Additional differences in amino acid sequence are noted at the N-terminal and C-terminal regions. For example, COX-2 lacks 17 amino acids in the N-terminus, but has an additional 18 amino acids in the C-terminus. These structural differences render the substrate binding site of COX-2 more accommodating of larger inhibitors (Fig. S1) than that of COX-1.

Inhibitory potencies of nonsteroidal antiinflammatory drugs (NSAIDs) rely heavily on the type of assay performed. The half maximal inhibitory concentration (IC<sub>50</sub>) values for COX-1 and COX-2 inhibitors do not indicate the mechanism of enzyme inhibition, and vary with substrate concentration, incubation time, and other assay-specific conditions. For this reason it is important to compare IC<sub>50</sub> values among inhibitors under identical assay conditions. COX-1 and COX-2 selective inhibitors operate through at least four types of mechanism including irreversible inhibition (e.g., aspirin), reversible competitive inhibition (e.g., ibuprofen), slow time-dependent reversible inhibition (e.g., indomethacin and flurbiprofen), and slow time-dependent irreversible inhibition (e.g., celecoxib and rofecoxib). Therefore, the selectivity (Data S1) observed by these small molecules (some shown in Fig. S1) is not determined simply by binding affinities, but is also partially attributed to enzyme kinetics.

## ACUTE SEIZURE THRESHOLD

Many studies have investigated the function of COX-2 in different seizure paradigms (Table S1). A majority of these studies used pentylenetetrazol (PTZ) to induce seizures in



**Figure 1.**  
Schematic of the COX-2 signaling cascade following initiation of status epilepticus.  
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rodents. For example, Dhir et al. (2006) examined the effects of COX-2 selective (rofecoxib, nimesulide) and non-selective inhibitors (aspirin, naproxen) on PTZ-induced convulsions. This study showed that oral administration of the COX-2 inhibitors in mice 45 min prior to an injection of a convulsion-inducing dose of PTZ (80 mg/kg) increased the mean onset time of clonus, reduced seizure duration, and speeded recovery to normal behavior and activity after PTZ seizures. Of interest, oral administration of celecoxib (2 mg/kg, but not 0.2 or 20 mg/kg) 60 min prior to injection of PTZ afforded an anticonvulsant action that was reversed by intracerebroventricular administration of PGE<sub>2</sub> (10 ng; Oliveira et al., 2008). If this finding can be repeated, it suggests that COX-2 activity may both facilitate and oppose seizure induction, and that facilitation requires activation of a PGE<sub>2</sub> receptor. The selective COX-2 inhibitors rofecoxib and nimesulide were both shown to be more effective than the nonselective inhibitors. Akula et al. (2008) investigated the effects of acute intraperitoneal (i.p.) injections of rofecoxib on the PTZ-induced seizure threshold in mice. A single intraperitoneal injection of rofecoxib (2 and 4 mg/kg but not 1 mg/kg) 45 min prior to PTZ increased the seizure threshold for the onset of all phases of PTZ-induced convulsions and significantly decreased the incidence of PTZ-induced convulsions. However, in a more recent study chronic oral administration of rofecoxib (30 mg/kg/day) for 5 days prior to PTZ was shown to have no effect on the incidence or severity of acute seizures induced by 40 or 55 mg/kg PTZ (Claycomb et al., 2011).

Pretreatment with COX-2 inhibitors dampens the development of kindling produced by PTZ in rats (Dhir et al., 2007) or rapid electrical stimulation in rats or mice (Take-miya et al., 2003; Tu & Bazan, 2003), observations very

likely explained by the aforementioned elevated seizure threshold by COX-2 inhibition. Conversely, administration of nimesulide or celecoxib to rats 1 h prior to kainic acid (KA) strongly augmented KA-induced seizures (Kunz & Oliw, 2001a; Gobbo & O'Mara, 2004). The KA seizures were more severe and prolonged in the inhibitor-treated group, and acute mortality was also increased. Recently, it was demonstrated that conditional genetic ablation of the COX-2 gene limited to principal forebrain neurons does not alter the latency to reach electrographic status epilepticus (SE) or seizure intensity following an intraperitoneal injection of pilocarpine compared to wild-type mice (Serrano et al., 2011), suggesting that the loss of COX-2 at least in these principal neurons does not alter the acute seizure threshold to pilocarpine. Together, these studies reveal some inconsistencies in regard to the role of COX-2 on acute seizures. In assessing the *in vivo* efficacy of a compound many factors should be considered such as the subject species, the convulsant, the vehicle of the test compound, the volume of fluid injected, and the timing and route of administration. Any of these factors can influence the consequence of pharmacologic manipulation of a target. Nevertheless, acute inhibition of COX-2 appears to be neutral or beneficial in most acute seizure models by increasing the acute seizure threshold. The corollary conclusion is that low constitutive expression of COX-2 in the brain reduces seizure threshold, which could be explained by the ability of PGE<sub>2</sub> to elevate neuronal excitability (Chen & Bazan, 2005).

## BLOOD-BRAIN BARRIER DISRUPTION

The blood-brain barrier (BBB) is a selectively permeable interface between the central nervous system (CNS) paren-

chyma, and the blood. Tight junctions between adjacent endothelial cells restrict traffic of materials in the blood to the brain and force molecules to be transported by specific transcellular routes that allow uptake of essential molecules but restrict harmful bacteria or large circulating molecules such as antibodies. Disruption of the BBB is a common feature in human temporal lobe epilepsy (van Vliet et al., 2007). Many reports show an association between seizure activity and BBB leakiness (Roch et al., 2002; Ballabh et al., 2004; Neuwelt, 2004; Seiffert et al., 2004; van Vliet et al., 2007; Serrano et al., 2011; Jiang et al., 2013). Some anticonvulsants including phenytoin have shown poor penetration into the brain of rodents when administered chronically in mice or rats (Potschka & Loscher, 2001; Rizzi et al., 2002; Loscher & Potschka, 2005). Up-regulation of p-glycoprotein (Pgp), which typically pumps drugs out of the brain, appears to underlie the development of resistance to phenytoin (Loscher & Potschka, 2005). Therefore, strategies for selective inhibition of the Pgp pump have been suggested to improve pharmacotherapy in epilepsy (Brandt et al., 2006; van Vliet et al., 2006).

A series of studies has been performed to identify the target cells and molecules that regulate Pgp expression. Pgp expression was detected in endothelial cells, astrocytes, microglia, and neurons (Decleves et al., 2000; Lee et al., 2001; Marroni et al., 2003), suggesting that antiepileptic drug resistance may be generated by the pumping of drugs from neuronal and nonneuronal cells into the blood or the interstitial fluid. Antiepileptic drugs (AEDs) themselves were suggested to be regulators of Pgp expression. Recently, three AEDs (phenobarbital, carbamazepine, and phenytoin) were shown to upregulate P-glycoprotein levels in capillary endothelial vessels in rats (Wen et al., 2008) and even more recently a study in mice provided *in vivo* evidence for the modulation of P-glycoprotein activity by AEDs (levetiracetam, topiramate, and phenytoin; Moerman et al., 2011). The upregulation of P-glycoprotein observed in isolated mouse and rat brain capillaries exposed to glutamate appears to be dependent on COX-2 signaling, as it is blocked by the selective COX-2 inhibitors celecoxib (Bauer et al., 2008), NS398, and indomethacin heptyl ester (Zibell et al., 2009). The COX inhibitors celecoxib (Zibell et al., 2009) and indomethacin (Bauer et al., 2008) also prevented seizure-induced P-glycoprotein upregulation in rat brain capillaries *in vivo* in the pilocarpine status epilepticus model. Likewise, the COX-2 inhibitors SC-58236 and NS-398 promoted brain delivery of phenytoin in epileptic rats (van Vliet et al., 2010) associated with downregulation of Pgp. Disruption of the BBB causes upregulation of COX-2 and subsequent induction of the Pgp in response to seizures (van Vliet et al., 2010). Further investigation revealed that pharmacologic inhibition of the prostaglandin E2 receptor EP1 by SC-51089 prevents seizure-associated Pgp upregulation at the BBB

(Pekcec et al., 2009), suggesting that blockade of the COX-2/EP1 signaling pathway could be a promising approach to control Pgp expression and to enhance access and efficacy of AEDs. However, it should be noted that in rats, inhibition of EP1 by SC-51089 failed to prevent pilocarpine-induced neurodegeneration in the hippocampal hilar formation (Pekcec et al., 2009).

Although direct inhibition of Pgp with tariquidar improves seizure control in rats treated with phenobarbital or phenytoin, pan inhibition of Pgp could disturb the protective nature of Pgp at the BBB leading to a deleterious effect (Brandt et al., 2006; van Vliet et al., 2006). An alternative would be to manipulate Pgp expression by regulating signaling molecules such as COX-2 that would not affect basal Pgp expression. Neuronal COX-2 induction and subsequent EP2 signaling coupled with BBB disruption may promote the epileptogenic process (Serrano et al., 2011; Jiang et al., 2013), which in turn leads to further upregulation of COX-2 (van Vliet et al., 2010). Even though studies targeting COX-2 suggest that COX-2 inhibition may be beneficial due to the role of this enzyme in epileptogenesis and pharmacotherapy, the adverse cardiovascular and cerebrovascular effects of COX-2 inhibitors severely limit these drugs as therapeutic agents.

## NEURODEGENERATION AND NEUROINFLAMMATION

COX-2 is an important mediator of neuroinflammation. In a pilocarpine mouse model, for example, either conditional ablation of the *COX-2* gene from principal forebrain neurons or postseizure administration of a brain-permeant EP2 receptor antagonist dampened the delayed cytokine burst in the hippocampus that follows SE (Serrano et al., 2011; Jiang et al., 2013). COX-2 activation in the brain has been shown to promote delayed neuronal damage in rodent models of temporal lobe epilepsy (Manabe et al., 2004; Kawaguchi et al., 2005; Takemiya et al., 2006; Polascheck et al., 2010; Serrano et al., 2011). Transgenic mice overexpressing neuronal COX-2 have increased sensitivity to glutamate excitotoxicity *in vitro* and *in vivo* (Kelley et al., 1999). Blocking the seizure-induced increase in COX-2 function with selective inhibitors such as rofecoxib reduced neuron death, suggesting a neuroprotective effect of COX-2 inhibitors (Kunz & Oliw, 2001b; Kawaguchi et al., 2005; Hewett et al., 2006; Takemiya et al., 2006; Polascheck et al., 2010), although this appears to depend on the severity of the seizure activity (Holtman et al., 2009) and the schedule of drug administration, with the greatest neuroprotection afforded through multiple doses with a selective COX-2 inhibitor administered *after* SE induction. Preseizure administration of COX-2 inhibitors either did not prevent neuronal death (Takemiya et al., 2006) or caused increased mortality following KA-induced seizures in rats (Kunz & Oliw, 2001a; Gobbo & O'Mara, 2004). A combination of

pretreatment and posttreatment with the nonselective COX inhibitor naproxen was neuroprotective in an NMDA excitotoxicity model (Silakova et al., 2004). Even when neuroprotection is observed after COX-2 inhibition, selective COX-2 inhibitors do not prevent epileptogenesis or reduce the frequency of spontaneous seizures in animal models of epilepsy (Holtman et al., 2009). These findings, taken together, suggest that the COX-2 signaling cascade, as a whole, is not required for epilepsy development, although it can be disease-modifying (Holtman et al., 2009; Polascheck et al., 2010). Similarly, other studies demonstrate that inhibition of COX-2 with celecoxib or SC-58236 *after* kainate-induced SE provided no significant improvement in neuronal damage or seizure activity (Gobbo & O'Mara, 2004; Holtman et al., 2009). By contrast, neuroprotection was observed following pretreatment of rofecoxib prior to administration of KA in rats (Kunz & Oliw, 2001b) or intrahippocampal injection of NMDA in mice (Hewett et al., 2006). The seemingly contradictory findings of the effect of COX-2 inhibitors on neurodegeneration and epileptogenesis in animal models might be due to the method of SE induction, the use of different COX-2 inhibitors with varied selectivity, variable treatment protocols and dosing schedules, as well as outcome measures (e.g., seizure intensity, cognitive deficits, cell death, and so on; Table S1). Nevertheless, COX-2 inhibition appears to be a potentially valuable therapeutic strategy for reducing seizure-related neuronal damage.

COX-2 knockout mice have proven useful in substantiating the effects demonstrated with pharmacologic inhibition of COX-2 before or after SE induction. Takemiya et al. (2006) reported that NS-398, a selective COX-2 inhibitor, administered repeatedly for 48 h after KA-induced seizures reduced hippocampal cell death, and this effect was mimicked in COX-2 knockout mice. Similarly, Manabe et al. (2004) demonstrated that NS-398 can significantly reduce the size of an NMDA-induced neocortical lesion. Furthermore, the lesion produced by NMDA in COX-2 knockout mice was significantly smaller than that in wild-type mice. Jarvela et al. (2011) used organotypic hippocampal slice cultures to determine the effect of COX-2 inhibition on KA-induced neuronal damage. Pretreatment with NS-398 did not prevent neuronal damage in this model. However, conditional ablation of the *COX-2* gene restricted to principal forebrain neurons protected mice against hippocampal neurodegeneration 4 days after pilocarpine-induced SE (Serrano et al., 2011).

Despite conflicting results using a variety of selective COX-2 inhibitors in different epilepsy models, there is ample evidence to suggest that seizure-induced COX-2 plays a role in epilepsy-related neurodegeneration. Further studies are necessary to elucidate the extent and pattern of COX-2 involvement in neuronal damage, and to explore the role of COX-2-related neuroinflammation in delayed neurodegeneration. A clearer picture might emerge by focusing

downstream in the COX-2 cascade. For example, EP2-receptor inhibition in the pilocarpine model is neuroprotective and completely recapitulates the protective effects of conditional ablation of COX-2 from forebrain neurons (Jiang et al., 2013), which points to a role for EP2 activation in seizure-induced neurodegeneration.

## SPONTANEOUS SEIZURE FREQUENCY IN THE CHRONIC STATE

COX-2 levels are increased in the brains of patients with epilepsy, especially temporal lobe epilepsy, and in animals that experience prolonged seizures (Desjardins et al., 2003; Serrano et al., 2011). Multiple studies using COX-2 inhibitors in animal models of epilepsy yielded controversial results regarding the involvement of COX-2 in spontaneous recurrent seizures (SRSs). For example, oral administration of the COX-2 inhibitor celecoxib (20 mg/kg) 1 day following a 1-h episode of SE induced by pilocarpine in rats, reduced the number of animals that had SRSs by 33%, reduced the frequency of observed behavioral seizures by 65%, and reduced seizure duration by 52%, as monitored by video recording during the light period from 28 to 42 days after SE (Jung et al., 2006). In another study, intraperitoneal injection of the COX-2 inhibitor parecoxib (10 mg/kg) twice daily for 17 days, starting immediately after the onset of a 90-min episode of pilocarpine-induced SE, had no effect on the incidence, frequency, or duration of SRSs, but reduced the severity of spontaneous seizures examined by continuous EEG/video monitoring (Polascheck et al., 2010). Treatment with another COX-2 selective inhibitor SC-58236 (10 mg/kg) by oral administration beginning 1 day before, shortly after, or 3–4 months after electrically induced SE in rats produced no antiepileptogenic or antiepileptic effect, but rather had severe adverse effects with a higher mortality after SE and after 2 weeks of chronic treatment (Holtman et al., 2009, 2010). This disparity in the effectiveness of COX-2 inhibition on SRSs might be related to differences in the duration of SEs experienced by the animals, by the dosing protocol of the COX-2 inhibitors (Table S1), or by off-target toxicity.

## COMORBIDITIES

Current cognitive comorbidities of epilepsy include attention deficit disorder (ADD), major depression, and anxiety. Because COX-2 is induced following seizure activity in the brain and depression is a comorbidity of epilepsy, it remains a question as to how COX-2 functions in patients who undergoing electroconvulsive seizure therapy for major depression. The function of COX-2 in major depression has been investigated in rodents, where COX-2 inhibitors alleviate memory loss following electroconvulsive seizures (Segi-Nishida, 2011). It would be worthwhile

exploring whether COX-2 inhibition is beneficial for patients who are undergoing electroconvulsive treatment (Segi-Nishida, 2011).

Under normal conditions, COX-2 is selectively expressed at moderate levels in several brain regions such as cerebral cortex, hippocampal CA3 region, and amygdala, likely a result of the activity dependence of its expression (Yamagata et al., 1993). Moreover, COX-2 resides in dendritic spines of glutamatergic neurons, where excitatory neurotransmission and synaptic plasticity occur (Kaufmann et al., 1996), suggesting a role for neuronal COX-2 in modulating synaptic activity and therefore cognitive functions (Yang & Chen, 2008). In hippocampal slices, a COX-2 inhibitor dampened both long-term potentiation (LTP) and long-term depression (LTD; Murray & O'Connor, 2003; Shaw et al., 2003). PGE<sub>2</sub>, but not PGD<sub>2</sub> or PGF<sub>2α</sub>, rescued the impaired long-term synaptic plasticity caused by COX-2 inhibition in hippocampal dentate granule neurons in vitro (Chen et al., 2002; Chen & Bazan, 2005), pointing to a potential role for one of the four PGE<sub>2</sub> receptors.

Constitutive COX-2 expression helps refine homeostatic synaptic functions in neurons, whereas the rapid induction of COX-2 following brain injuries leads to neuropathologies presumably also through its involvement in synaptic modification (Yang & Chen, 2008). Indeed, a number of animal studies using selective COX-2 inhibitors indicate that COX-2 plays essential roles in spatial learning and memory (Teather et al., 2002; Rall et al., 2003; Shaw et al., 2003; Sharifzadeh et al., 2005). Cognitive impairments are often associated with epilepsy, especially temporal lobe epilepsy, when seizures initiate and propagate within the critical memory structures of the medial temporal lobe such as hippocampus and amygdala (LaFrance et al., 2008). Cognitive deficits are also commonly evidenced in animal models of epilepsy. For example, 9–12 weeks following pilocarpine-induced SE, mice learned the escape platform location of the Morris water maze (MWM) task significantly more slowly than their nonseizure cohorts (Muller et al., 2009). Administration of the COX-2 inhibitor rofecoxib for 2 days after KA had no effect on behavior in the MWM task (Kunz et al., 2005). Similarly, the COX-2 inhibitor parecoxib did not affect the behavioral and cognitive alterations associated with epilepsy (Polascheck et al., 2010). On the other hand, conditional COX-2 ablation in principal forebrain neurons reduced the latency to find a previously learned target hole in a retrograde amnesia task in mice that had experienced pilocarpine-induced SE 1 month earlier, which was a selective effect in that their motor behavior and other cognitive measures were not changed (Levin et al., 2012).

Of interest, celecoxib administered after but not prior to KA injection significantly reduced the learning and object exploration deficits in a MWM task (Gobbo & O'Mara, 2004). As a first-generation COX-2 inhibitor, celecoxib has a much lower selectivity for COX-2 over COX-1 compared to rofecoxib and parecoxib, thus a COX-2 independent

action cannot be excluded in this study. Nonetheless, suppression of COX-2 activity by selective inhibitors or genetic manipulation reduces COX-2-promoted pathologies in the brain, including neuroinflammation and neurodegeneration, and this might potentially underlie reduced seizure-triggered cognitive deficits. However, the possibility that rofecoxib might also block the benefits of basal COX-2 in synaptic plasticity, learning, and memory during the period of recovery should be considered. Further studies on the dosing of COX-2 selective inhibitors in combination with other antiepileptic therapies would be needed to reevaluate COX-2 as a therapeutic target to improve cognitive functions following seizure attacks (see Data S1). The downstream signaling molecules in the COX-2 cascade can serve as alternative therapeutic targets for reducing cognitive deficits following prolonged seizures.

## CONCLUSIONS AND FUTURE DIRECTION

Cyclooxygenase-2 is known to play a key role in the early inflammatory response to an insult, and consequently a significant role in postseizure inflammation and hyperexcitability of the brain. Most investigators report that pretreatment of animals with a COX-2 inhibitor *before* a convulsant stimulus can dampen seizure intensity, which would be consistent with the excitatory effect of PGE<sub>2</sub> generated by constitutively active COX-2. *Postseizure* treatment, on the other hand, can be neuroprotective as expected from the strong proinflammatory role of induced COX-2. Although induction of this enzyme may be beneficial in the very early postseizure period, delayed and prolonged induction appears to lead to damaging long-term consequences. To date, none of the selective COX-2 inhibitors tested has reproducibly slowed epilepsy disease progression as measured by the appearance of spontaneous seizures.

Potential complications from chronic exposure to high doses of COX-2 inhibitors include nonselective inhibition of COX-1, an enzyme that can mediate beneficial effects, and suppression of the physiologic regulation of synaptic plasticity by basal COX-2. Therefore, the dose and administration pattern may be critical to achieve beneficial effects of COX-2 inhibitors. The subject species, dosing amount, and therapeutic window should all be explored with attention and reference to the induction pattern of COX-2. The risk of serious cardiovascular side effects that accompany chronic COX-2 inhibition makes it unlikely that *chronic* COX-2 inhibition is a viable therapeutic strategy, although short-term exposure might be useful.

An alternative therapeutic direction to COX-2 inhibitors would involve the downstream effector molecules in the COX-2 signaling cascade (i.e., prostanoid synthases and receptors), which should offer more selective targets for disease modification. Recently, attention has been given to such molecules. For example, the EP2 receptor appears to

contribute to the development of neuropathologies following status epilepticus. The discovery of selective prostanoid receptor modulators may help avoid the complication of serious side effects by chronic inhibition of COX-2. Perhaps it is now time to switch the focus and effort from COX-2 to the key downstream players in the COX-2 cascade such as the prostanoid synthases and receptors, which offer more selective therapies to pathway specific components of neurologic diseases. Modulators of prostanoid receptor function could also improve our understanding of the role of COX-2 in the brain immune response and inflammation, and consequently the effects of COX-2 on the epileptic patient. This information will aid future research as well as the development of new treatment methods and medications.

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## DISCLOSURE

There are no conflicts of interest in relation to this work. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this manuscript is consistent with the Journal's guidelines.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Data on the selectivity of COX-2 inhibitors and a possible therapy involving the combination of selective COX-2 inhibitors and antiepileptic drugs to treat epilepsy.

**Table S1.** Effects of COX-2 inhibitors on neuropathologies in rodent models of epilepsy.

**Figure S1.** Nonsteroidal antiinflammatory drugs: Structures **1–5** are nonselective, but COX-1 preferring inhibitors. Structures **6–10** are selective COX-2 inhibitors. Drugs **7–8** have been withdrawn from the United States market, due to fatal cardiovascular side effects.