



Mechanisms of Seizure-Induced Inflammation of the Brain: Many Possible Roles for Neuronal COX-2

Ablation of Cyclooxygenase-2 in Forebrain Neurons is Neuroprotective and Dampens Brain Inflammation after Status Epilepticus.

Serrano GE, Lelutiu N, Rojas A, Cochi S, Shaw R, Makinson CD, Wang D, Fitzgerald GA, Dingledine R. *J Neurosci* 2011;31(42):14850–14860.

Cyclooxygenase-2 (COX-2), a source of inflammatory mediators and a multifunctional neuronal modulator, is rapidly induced in select populations of cortical neurons after status epilepticus. The consequences of rapid activity-triggered induction of COX-2 in neurons have been the subject of much study and speculation. To address this issue directly, we created a mouse in which COX-2 is conditionally ablated in selected forebrain neurons. Results following pilocarpine-induced status epilepticus indicate that neuronal COX-2 promotes early neuroprotection and then delayed neurodegeneration of CA1 pyramidal neurons, promotes neurodegeneration of nearby somatostatin interneurons in the CA1 stratum oriens and dentate hilus (which themselves do not express COX-2), intensifies a broad inflammatory reaction involving numerous cytokines and other inflammatory mediators in the hippocampus, and is essential for development of a leaky blood-brain barrier after seizures. These findings point to a profound role of seizure-induced neuronal COX-2 expression in neuropathologies that accompany epileptogenesis.

Small Molecule Antagonist Reveals Seizure-Induced Mediation of Neuronal Injury by Prostaglandin E2 Receptor Subtype EP2.

Jiang J, Ganesh T, Du Y, Quan Y, Serrano G, Qui M, Spiegel I, Rojas A, Lelutiu N, Dingledine R. [Published ahead of print on February 12 2012, *Proc Natl Acad Sci USA*.]

With interest waning in the use of cyclooxygenase-2 (COX-2) inhibitors for inflammatory disease, prostaglandin receptors provide alternative targets for the treatment of COX-2-mediated pathological conditions in both the periphery and the central nervous system. Activation of prostaglandin E2 receptor (PGE2) subtype EP2 promotes inflammation and is just beginning to be explored as a therapeutic target. To better understand physiological and pathological functions of the prostaglandin EP2 receptor, we developed a suite of small molecules with a 3-aryl-acrylamide scaffold as selective EP2 antagonists. The 12 most potent compounds displayed competitive antagonism of the human EP2 receptor with K_B 2–20 nM in Schild regression analysis and 268- to 4,730-fold selectivity over the prostaglandin EP4 receptor. A brain-permeant compound completely suppressed the up-regulation of COX-2 mRNA in rat cultured microglia by EP2 activation and significantly reduced neuronal injury in hippocampus when administered in mice beginning 1 h after termination of pilocarpine-induced status epilepticus. The salutary actions of this novel group of antagonists raise the possibility that selective block of EP2 signaling via small molecules can be an innovative therapeutic strategy for inflammation-related brain injury.

Commentary

It is well established that seizures, particularly status epilepticus (SE), induce upregulation of cyclooxygenase-2 (COX-2) in both neurons and non-neuronal cells (1, 2). COX-2 plays a role in seizure-induced leukocyte infiltration, astrogliosis, microglial activation, and breakdown of the blood-brain barrier (BBB);

thus, COX-2 is clearly an important component of brain inflammation. COX-2 is also thought to play an important role in seizure-induced neurodegeneration; in fact, COX-2 inhibitors generally reduce neuronal death and provide neuroprotection. Although COX-2 is thought to be expressed primarily in non-neuronal cells, it is also expressed in neurons; furthermore, seizures strongly enhance neuronal COX-2 (nCOX-2) expression. An important question addressed by the present papers is the role of nCOX-2 in brain inflammatory mechanisms, which was studied in a mouse model in which COX-2 was conditionally ablated in specific forebrain neurons. One important

Epilepsy Currents, Vol. 12, No. 3 (May/June) 2012 pp. 115–117
© American Epilepsy Society

OPEN ACCESS Freely available online



finding is that neuron-specific conditional COX-2 knock-out mice (nCOX-2 cKO) showed less neuronal death than wild-type controls, which suggests that nCOX-2 contributes to seizure-induced neurodegeneration. A less expected and maybe more important outcome was prevention of BBB opening. This latter result provides the beginning of a molecular understanding of how neurons control the endothelial cells and astrocytes that form the BBB.

One hypothesis investigated in these studies is that nCOX-2 has a biphasic effect on seizure-induced neuronal death. In an initial set of studies, the properties and consequences of pilocarpine-induced repetitive seizures (i.e., SE) were unaltered in the nCOX-2 cKO mice, which allowed investigation of the effects of SE on subsequent neuronal death. The data, which focused upon hippocampal CA1 pyramidal cells as the principal neurons, suggest that COX-2 mediates an early form of neuroprotection, which is then followed after a delay by neurodegeneration. This interpretation is based primarily on neuronal counts (e.g., FluoroJade) at 1 day versus 4 days after SE. As the authors point out, the initial neuroprotective effect was not statistically significant and was only a trend, and there was a more robust neurodegenerative effect at the 4-day time point. Although these data suggest a neuroprotective effect, the authors make cautionary statements that deserve attention, and they emphasize the difficulty in quantitatively assessing the effects of SE on neuronal death, given the substantial variability from animal to animal. The possibility that COX-2 and other molecules in inflammatory pathways have biphasic effects on neuronal survival/death deserves additional investigation; validation of this result would add additional complexity to this already complicated pathway.

Somatostatin-expressing GABAergic interneurons are known to be highly sensitive to repetitive seizures, and their loss is a common feature of epileptic tissue in both animal models and resected samples from humans with intractable temporal lobe epilepsy. The nCOX-2 cKO mice showed preservation of somatostatin-expressing interneurons after pilocarpine-induced SE, unlike the wild-type mice. Therefore, although COX-2 did not appear to be expressed in GABAergic interneurons, nCOX-2 surprisingly appeared to be involved in their degeneration. This suggests that COX-2 expression in principal cells, such as CA1 pyramidal cells and dentate granule cells, somehow plays a role in the seizure-induced degeneration of GABAergic interneurons. This observation leads to a new level of complexity in the inflammatory response to repetitive seizures, because these interneurons are one of the few cell types that apparently do not express COX-2. The mechanism by which nCOX-2 contributes to—or even mediates—sensitivity to repetitive seizures in these interneurons also deserves more study.

An important product of COX-2 is prostaglandin E2. This prostaglandin activates four different receptors, EP1 through EP4. Other evidence has already suggested that activation of EP2 is neuroprotective (3). In pilocarpine-treated rats, intraventricular injection of butaprost, a selective EP2 agonist, immediately after termination of SE reduced the number of FluoroJade-stained neurons by 50%. These data support the hypothesis that EP2 receptors are important in nCOX-2-mediated neurodegeneration. Recent data show that delayed

administration of an EP2 antagonist, timed to match the peak of COX-2 induction (approximately 5 hours after the seizures), is strongly neuroprotective when examined 4 days after SE. Thus, EP2 receptor modulators, similar to other anti-inflammatory compounds (4), may be efficacious neuroprotective drugs.

The field of brain inflammation continues to show increasing levels of complexity, but how this will be used to suppress epileptogenesis after a brain injury or treat pharmacoresistant epilepsy remains unclear. When considering global inhibition of COX-2 by systemic administration of inhibitors, data from several laboratories point to a role in seizure-induced neurodegeneration. The degree to which neuronal death leads to inflammation, or inflammation contributes to neuronal death, or both needs additional clarification, particularly in regard to the mechanisms of epileptogenesis. For example, an *in vitro* model of epileptogenesis that is based on the damage inherent in preparation of organotypic slices has little or no inflammatory response but consistently develops robust spontaneous recurrent seizure-like events in a time-dependent manner (5). Although injury-induced epileptogenesis can occur *in vitro* with little or no inflammatory response, inflammation may still contribute to epileptogenesis in the intact animal or human. One could speculate that the severity and type of inflammation (i.e., which cytokines are released, by which cells, and when) may influence the amount of neuronal death, which then secondarily affects epileptogenesis and/or its rate of progression.

What is needed is the determination of whether experimental manipulation of inflammatory mechanisms after a brain injury (e.g., severe SE) affects the time-dependent development of spontaneous recurrent seizures in an animal model of acquired epilepsy (i.e., disease modification). If inflammatory mechanisms are important in the generation of the spontaneous recurrent seizures that define epilepsy, experimental manipulation of these mechanisms after a brain insult ought to alter the frequency and severity of the spontaneous seizures. In fact, if disruption of the BBB and associated inflammatory mechanisms are important in epilepsy, a direct investigation of the role of anti-inflammatory agents in the development of chronic epilepsy and the maintenance of spontaneous epileptic seizures in patients with acquired epilepsy ought to be no more difficult (and ought to be associated with no more risk) than a standard clinical trial of an antiepileptic drug. One difficulty is that inflammation is multidimensional, with beneficial as well as detrimental consequences. An analogy with infection might be appropriate: The inflammatory process that causes chemotaxis of immune cells to the site of infection is initially beneficial in scavenging the invading bacteria, but prolonged inflammation with consequent tissue swelling can cause structural changes that impair normal function.

by F. Edward Dudek, PhD

References

1. Friedman A, Dingledine R. Molecular cascades that mediate the influence of inflammation on epilepsy. *Epilepsia* 2011;52(suppl):33–39.



2. Walker L, Sills GJ. Inflammation and epilepsy: The foundations for a new therapeutic approach in epilepsy? *Epilepsy Curr* 2012;12:8–12.
3. McCullough L, Wu L, Haughey N, Liang X, Hand T, Wang Q, Breyer RM, Andreasson K. Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. *J Neurosci* 2004;24:257–268.
4. Vezzani A, Bartfai T, Bianchi M, Rossetti C, French J. Therapeutic potential of new antiinflammatory drugs. *Epilepsia* 2011;52(suppl):67–69.
5. Berdichevsky Y, Dzhala V, Mail M, Staley KJ. Interictal spikes, seizures and ictal cell death are not necessary for post-traumatic epileptogenesis in vitro. *Neurobiol Dis* 2012;45:774–785.



American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.

Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Other relationships

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.



American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Section #1 Identifying Information

1. Today's Date: 5-7-12
2. First Name Edward Last Name Dudek Degree PhD
3. Are you the Main Assigned Author? Yes No

If no, enter your name as co-author:

4. Manuscript/Article Title: Mechanisms of Seizure-Induced Inflammation of the Brain: Many Possible Roles for Neuronal COX-2
5. Journal Issue you are submitting for:

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship just add rows to this table.

Type	No	Money Paid to You	Money to Your Institution*	Name of Entity	Comments**
1. Grant	<input type="checkbox"/>				
2. Consulting fee or honorarium	<input type="checkbox"/>				
3. Support for travel to meetings for the study or other purposes	<input checked="" type="checkbox"/>				
4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like	<input checked="" type="checkbox"/>				
5. Payment for writing or reviewing the manuscript	<input checked="" type="checkbox"/>				
6. Provision of writing assistance, medicines, equipment, or administrative support.	<input checked="" type="checkbox"/>				
7. Other	<input type="checkbox"/>				

* This means money that your institution received for your efforts on this study.

** Use this section to provide any needed explanation.

Section #3 Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

Type of relationship (in alphabetical order)	No	Money Paid to You	Money to Your Institution*	Name of Entity	Comments**
1. Board membership	<input type="checkbox"/>	<\$1,000		Equity in Epitel, Inc.	
2. Consultancy	<input type="checkbox"/>	\$40,000.00		Neurotherapeutics Pharma, Epitel, Inc.	
3. Employment	<input checked="" type="checkbox"/>				
4. Expert testimony	<input checked="" type="checkbox"/>				
5. Grants/grants pending	<input type="checkbox"/>		\$900,000.00	NINDS, Neurotherapeutics Pharma, Epitel, Inc. and Johnson	
6. Payment for lectures including service on speakers bureaus	<input type="checkbox"/>				
7. Payment for manuscript preparation.	<input checked="" type="checkbox"/>				
8. Patents (planned, pending or issued)	<input type="checkbox"/>		\$1,100,000	University of Utah	
9. Royalties	<input checked="" type="checkbox"/>				
10. Payment for development of educational presentations	<input checked="" type="checkbox"/>				
11. Stock/stock options	<input checked="" type="checkbox"/>			Equity in Epitel, Inc.	
12. Travel/accommodations/meeting expenses unrelated to activities listed.**	<input checked="" type="checkbox"/>				
13. Other (err on the side of full disclosure)	<input checked="" type="checkbox"/>				

* This means money that your institution received for your efforts.

** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section #4 Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- No other relationships/conditions/circumstances that present a potential conflict of interest.
- Yes, the following relationships/conditions/circumstances are present:

Thank you for your assistance.
Epilepsy Currents Editorial Board