Inhibition of the prostaglandin receptor EP2 following status epilepticus reduces delayed mortality and brain inflammation

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Prostaglandin E2 is now widely recognized to play critical roles in brain inflammation and injury, although the responsible prostaglandin receptors have not been fully identified. We developed a potent and selective antagonist for the prostaglandin E2 receptor subtype EP2, TG6-10-1, with a sufficient pharmacokinetic profile to be used in vivo. We found that in the mouse pilocarpine model of status epilepticus (SE), systemic administration of TG6-10-1 completely recapitulates the effects of conditional ablation of cyclooxygenase-2 from principal forebrain neurons, namely reduced delayed mortality, accelerated recovery from weight loss, reduced brain inflammation, prevention of blood–brain barrier opening, and neuroprotection in the hippocampus, without modifying seizures acutely. Prolonged SE in humans causes high mortality and morbidity that are associated with brain inflammation and injury, but currently the only effective treatment is to stop the seizures quickly enough with anticonvulsants to prevent brain damage. Our results suggest that the prostaglandin receptor EP2 is critically involved in neuroinflammation and neurodegeneration, and point to EP2 receptor antagonism as an adjunctive therapeutic strategy to treat SE.

inflammatory cytokine | electroencephalography | epileptogenesis | gliosis | neuronal injury

As a dominant product of cyclooxygenase-2 (COX-2 or PTGS2) in the brain, prostaglandin E2 (PGE_2) is emerging as a crucial mediator of many COX-2–driven pathological events in the central nervous system (CNS) (1). PGE_2 acts on four G protein-coupled receptors named EP1, EP2, EP3, and EP4. Among these, the EP2 receptor is widely expressed in the brain and plays important physiologic functions, such as in neuronal plasticity (2, 3). However, recent studies have identified a possible link between EP2 signaling and secondary neurotoxicity in models of chronic inflammation and neurodegeneration (1, 4–6). In a rodent model of amyotrophic lateral sclerosis, for example, EP2 receptor knockout mice exhibit improved survival, down-regulation of proinflammatory enzymes, and reduced oxidative stress (6).

Prolonged status epilepticus (SE) in humans is associated with brain injury and substantial morbidity. Mortality is high during refractory SE that requires general anesthesia (7, 8), and the 30-d mortality is about 35–37% for adults who experience at least 60 min of SE (9). Outcome in humans is dependent upon age, etiology, and SE duration (8–10), and currently the only effective treatment is to stop the seizures quickly enough to prevent brain damage (10). Most deaths from nonrefractory SE occur in the 2-wk period after successful treatment rather than during the seizure episode itself (9), pointing to a delayed but cascading set of responsible events. In mice, prolonged SE induced by pilocarpine causes >25% delayed mortality (11), and is associated with a series of molecular and cellular events in the brain, including neurodegeneration, and selective inflammatory reactions involving reactive microglia and astrocytes (12). Although the underlying cellular and molecular mechanisms are incompletely understood, it is known that the rapidly up-regulated COX-2 after seizures promotes brain inflammation and secondary neurodegeneration (13–19). We recently showed that COX-2 of neuronal origin was responsible for these effects (19). However, which of the nine prostanoid receptors (1) mediates SE-induced inflammation and neurodegeneration is not fully understood.

We hypothesize that EP2 receptor activation contributes to neuronal injury and associated morbidities following SE. We reported previously that brief exposure of mice to a modestly brain-permeable EP2 antagonist soon after pilocarpine-induced SE reduced early neurodegeneration in the hippocampus as assessed 24 h later (20). Here we report that administration of an EP2 antagonist with improved pharmacokinetic properties in mice beginning 4 h after pilocarpine-induced SE produced a broad range of beneficial effects, including a sharp reduction in delayed mortality and neuroinflammation through a mechanism of action that does not involve acute anticonvulsant effects. Recognizing the untoward consequences of COX-2 inhibitors (21), EP2 receptor inhibition could be an appealing therapeutic strategy to reduce brain inflammation and injury following SE.

Results

Development and Characterization of EP2 Receptor Antagonist. We previously reported a prostaglandin receptor EP2 antagonist, TG4-155, with a relatively short plasma half-life (~0.6 h) and low brain:plasma ratio (~0.3) after systemic administration in mice (20, 22). We recently created an analog compound, TG6-10-1 (Fig. S1*A*), which has a superior pharmacokinetic profile making it suitable for more extensive testing. EP2 receptor activation by PGE_2 stimulates adenylate cyclase to elevate cytoplasmic cAMP level. We used a time-resolved fluorescence resonance energy transfer assay to monitor PGE_2-induced cAMP accumulation in C6 glioma (C6G) cells overexpressing human EP2 receptor (23). The potency of compound TG6-10-1 was evaluated by its effects on the concentration–response curves of PGE_2 in C6G-EP2 cells. Cells were incubated first with vehicle, 0.01, 0.1, 1, or 10 μM TG6-10-1 for 10 min, and then with increasing concentrations of PGE_2 for 40 min to activate the EP2 receptor. TG6-10-1 produced concentration-dependent, parallel rightward shifts in the PGE_2 concentration–response curve without affecting the maximal response (Fig. S1*B*). Schild regression analysis indicated that TG6-10-1 has a competitive mechanism of antagonism of the EP2 receptor with an equilibrium dissociation constant for the antagonist-receptor complex (K_B) of 17.8 nM (Fig. S1*C*).

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The selectivity of TG6-10-1 was evaluated against other prostanoid receptors in cell-based functional assays. In a comparison of Schild K_B values, TG6-10-1 displayed at least 300-fold selectivity for the EP2 receptor over human EP3, EP4, and IP receptors, 100-fold selectivity against human EP1, 25-fold selectivity against human FP and TP, and 10-fold selectivity against human DP1 receptors (Fig. S1*D*). These results indicate that of the eight canonical prostanoid receptors, TG6-10-1 shows low-nanomolar antagonist activity against only EP2 and DP1, the receptor activated by prostaglandin D2 (PGD_2). In addition, other off-target activity assays showed that TG6-10-1 had negligible effect on a panel of 40 enzymes, ion channels, receptors, and neurotransmitter transporters (IC_{50}s > 10 μM), except that TG6-10-1 weakly inhibited the serotonin 5-hydroxytryptamine 2B (5-HT2B) receptor with IC_{50} = 7.5 μM (Table S1). At a high concentration (10 μM), TG6-10-1 had little or no effect on the enzymatic activity of COX-1 (7% inhibition) and COX-2 (14% inhibition), and inhibited the leukotriene B4 (LTB_4) receptor BLT1 by 1% (Table S1). TG6-10-1 displayed a plasma half-life of ~1.6 h and a brain:plasma ratio of 1.6 after systemic administration (5 mg/kg, i.p.) in mice (Fig. S1*E*). Thus, compared with the original compound (20, 22), the redesigned compound TG6-10-1 has much improved pharmacokinetic properties, which justifies its use for in vivo study.

EP2 Receptor Antagonist Reduces Delayed Mortality, Accelerates Recovery from Weight Loss, and Improves Functional Recovery of Mice After Status Epilepticus. COX-2 is rapidly up-regulated in selected forebrain neurons after a seizure (13, 24), and ablation of the COX-2 gene restricted to these neurons brings a broad range of beneficial effects in mice that had experienced SE (11, 19). We investigated the effect of EP2 receptor inhibition by TG6-10-1 after pilocarpine-induced SE in C57BL/6 mice. SE was allowed to proceed for 1 h and then terminated by pentobarbital. Three hours later (i.e., 4 h after SE onset), vehicle or TG6-10-1 was administered (5 mg/kg, i.p.). Two additional doses of TG6-10-1 were administered at 21 and 30 h after SE onset (Fig. 1*A*) to approximately match the temporal pattern of COX-2 induction after pilocarpine. All in vivo studies used this treatment protocol. Given TG6-10-1 pharmacokinetics (Fig. S1*E*), its brain concentration should be more than twice its EP2 receptor K_B for at least 5 h following each injection, but TG6-10-1 should inhibit DP1 for less than 1 h following injection. None of the other prostanoid receptors is sensitive enough to TG6-10-1 to be appreciably inhibited by this dose. Thus, most of the effects of TG6-10-1 on prostanoid receptors can be attributed to inhibition of EP2, although DP1 can potentially contribute.

Substantial delayed mortality occurs in the week following SE in mice and humans (9, 11). A significant increase in survival was observed in post-SE mice that received TG6-10-1 compared with those in the vehicle group (Fig. 1*B*). Administration of TG6-10-1 improved 1-wk survival from 60 to 90% after SE (P = 0.029; Fig. 1*B*). SE also caused substantial weight loss of the animals, which is gradually regained over a week in vehicle-treated mice. TG6-10-1 accelerated the recovery of lost weight (P < 0.01 at day 4; Fig. 1*C*). About half of the mice (9 of 17) that received vehicle continued weight loss between days 1 and 4 after SE, whereas only 1 of 17 TG6-10-1–treated mice lost weight during the same period (P < 0.01; Fig. 1*D*). Weight gain by mice experiencing SE followed by TG6-10-1 treatment approximated that of control mice that had not experienced SE (Fig. 1*D*). Four days after SE, all 17 animals that received TG6-10-1 showed normal behavior, such as locomotion, drinking, eating, and nest building, whereas 5 of 17 (29%) of the animals receiving vehicle were not able to build nests by day 4 (P < 0.05; Fig. 1*E*). Nesting is a sensitive indicator of brain lesions, especially in the hippocampal area (25). Taken together, these results demonstrate that administration of the EP2 antagonist with the schedule shown in Fig. 1*A* improved survival, accelerated recovery of lost weight, and improved functional recovery following SE.

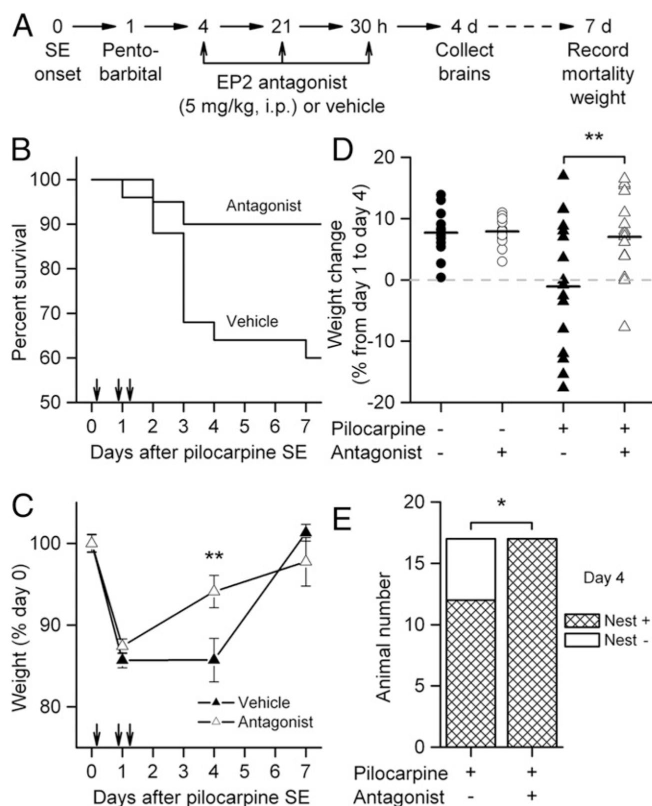

Fig. 1. EP2 receptor antagonist reduces mortality and weight loss and accelerates functional recovery after SE. (*A*) Animal experimental procedure. Mice were injected with pilocarpine (280 mg/kg, i.p.) and after 60 min of SE were injected with pentobarbital (30 mg/kg, i.p.) to terminate SE. Three hours later (i.e., 4 h after SE onset), mice were injected with EP2 antagonist TG6-10-1 (5 mg/kg, i.p.) or vehicle; each mouse received two additional doses of TG6-10-1 at 21 and 30 h after SE onset. This protocol was used for all in vivo experiments. Brains of a group of mice were collected for neuropathological studies 4 d after SE. (*B*) Survival rates of animals that received vehicle (n = 25) or TG6-10-1 (n = 20) up to day 7 after SE (P = 0.029, Kaplan–Meier survival analysis). The arrows indicate times of administration of TG6-10-1. (*C*) Effect of TG6-10-1 on mouse weight change after SE (n = 7–31, **P < 0.01, two-way ANOVA with post hoc Bonferroni test). Data are shown as mean ± SEM. (*D*) Weight change of individual animals from day 1 to day 4 after SE (n = 14–17, **P < 0.01, Fisher's exact test). (*E*) Nest construction behavior of animals at day 4 post-SE (n = 17, *P < 0.05, Fisher's exact test).

EP2 Antagonist Reduces SE-Induced Brain Inflammation and Blood–Brain Barrier Opening. Brain inflammation is a prominent consequence of seizure-promoted neuronal COX-2 induction (19). To investigate the possibility that EP2 receptor activation promotes brain inflammation after SE, we measured the mRNA levels of three inflammatory cytokines (IL-1β, IL-6, TNF-α), four chemokines (CCL2, CCL3, CCL4, CXCL10), and four markers of gliosis (astrocytes: GFAP and S100B; microglia: Iba1 and CD11b) in hippocampi 4 d after SE. Mice received either vehicle or EP2 antagonist TG6-10-1 in three doses as described above (Fig. 1*A*). All seven cytokines and chemokines tested were substantially induced by SE, and administration of TG6-10-1 significantly blunted their induction by an average of 54% (P < 0.05; Fig. 2*A*); likewise, induction of mRNA encoding the four glial proteins was reduced by an average of 43% (P < 0.05; Fig. 2*B*), which was confirmed by immunostaining (Fig. 2*C*). These findings support an involvement of EP2 receptor activation in seizure-induced brain inflammation.

The blood–brain barrier becomes more permeable during inflammation in various neuropathological conditions, including

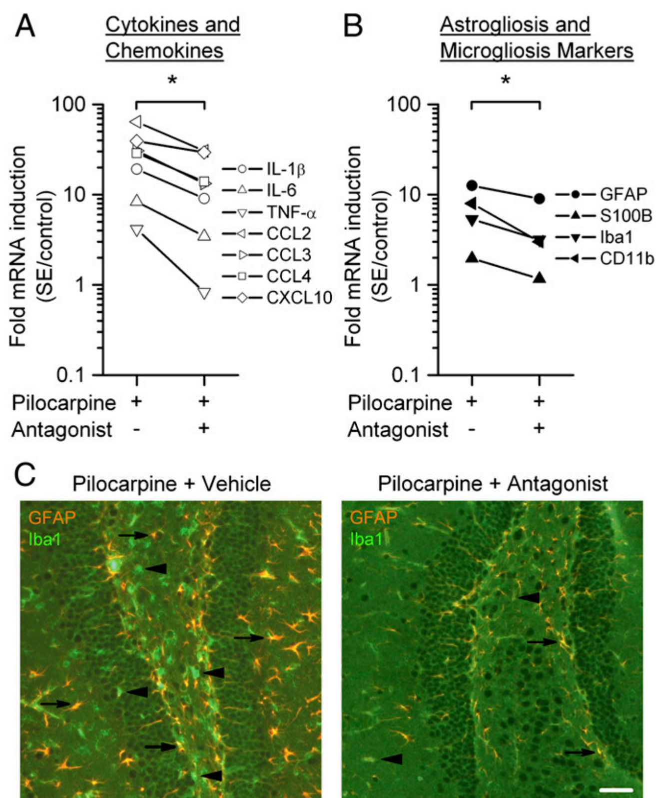

Fig. 2. EP2 receptor antagonist relieves brain inflammation after SE. (*A*) mRNA levels of seven cytokines and chemokines in mouse hippocampi were measured by quantitative real-time PCR. All tested cytokines and chemokines were substantially induced 4 d after pilocarpine-induced SE, as shown by fold mRNA induction, which was calculated as the mean mRNA level in SE mice compared with that in control mice without SE. Administration of EP2 antagonist TG6-10-1 significantly blunted SE-induced mRNA induction (n = 8–9, $*P < 0.05$, two-tailed paired t test). (*B*) Similar results were obtained with markers of astroglial (GFAP and S100B) or microglial (Iba1 and CD11b) activation (n = 8–9, $*P < 0.05$, two-tailed paired t test). (*C*) Hippocampal GFAP and Iba1 staining for activated astrocytes (arrows) and microglia (arrowheads), respectively, in mice treated with vehicle (*Left*) or TG6-10-1 (*Right*) 4 d after SE. (Scale bar, 50 μm.)

seizures (26, 27). Opening of the blood–brain barrier was demonstrated in humans with epilepsy and rats after seizures by measuring the extravasation of serum albumin into the brain parenchyma (28). In our hands, serum albumin was found in the cortex 4 d after SE as measured by Western blot analysis ($P < 0.01$; Fig. 3 *A* and *B*), a finding consistent with blood–brain barrier breakdown following pilocarpine-induced SE. Extravasation of albumin into the brain after SE appears to promote the progression of epilepsy, because injection of albumin directly into the brain enhances inflammation and intensifies subsequent development of spontaneous seizures (29). Interestingly, TG6-10-1 nearly eliminated serum albumin leakage into the cortex 4 d after SE ($P < 0.05$; Fig. 3 *A* and *B*), which suggests that EP2 receptor activation is necessary for blood–brain barrier opening following SE. Note that mice were perfused with saline to displace the blood from brain vessels, and thus the residual albumin in cortices from control mice derives from the cerebrospinal fluid (30).

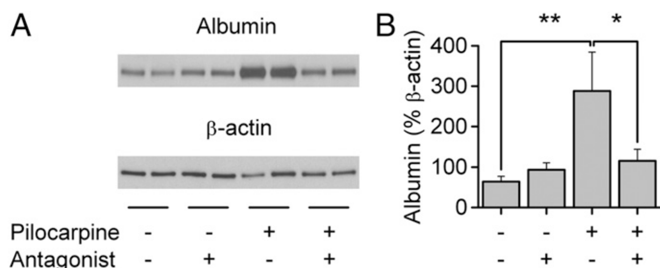

Fig. 3. EP2 receptor antagonist maintains the integrity of the blood–brain barrier after SE. (*A*) Serum albumin leak into the cortex 4 d after SE was used to assess the integrity of the blood–brain barrier. The albumin protein levels in cortices of control or SE mice that received vehicle or EP2 antagonist TG6-10-1 were measured by Western blot with β-actin as loading control. (*B*) Normalized band intensity of the albumin protein (n = 3–6, $*P < 0.05$, $**P < 0.01$, one-way ANOVA and post hoc Bonferroni test with selected pairs).

EP2 Antagonist Reduces Neurodegeneration in Hippocampus. We next evaluated neurodegeneration in hippocampi from mice that received TG6-10-1 or vehicle at 4, 21, and 30 h after SE and were sacrificed on day 4. Coronal brain sections were stained with Fluoro-Jade (0.001%, wt/vol), and the number of Fluoro-Jade–positive cells in hippocampal subregions cornu ammonis area 1 (CA1), CA3, and dentate hilus was determined. Pilocarpine-induced SE caused substantial hippocampal neurodegeneration in vehicle-treated mice 4 d after SE (Fig. 4*A*), whereas no positive staining was detected in mice treated with vehicle or TG6-10-1 alone rather than pilocarpine (Fig. S2). Systemic administration of TG6-10-1 reduced the SE-induced neurodegeneration score by 66% ($P < 0.05$) in CA1 and by 52% in CA3, and reduced cell loss in hilus by 55% ($P < 0.01$) (Fig. 4*B*). These results support the conclusion that EP2 receptor activation promotes neuronal death following SE.

EP2 Antagonist Is Not an Acute Anticonvulsant in the Pilocarpine SE Model. The benefits of EP2 inhibition after SE described above might be caused by a direct anticonvulsant effect. We did two experiments to examine this possibility. First, mice received vehicle or TG6-10-1 (5 mg/kg, i.p.) followed 1 h later by pilocarpine (280 mg/kg, i.p.), and behavioral seizures were scored on a seven-point scale (12). Pretreatment with TG6-10-1 did not change either the temporal evolution of behavioral seizures following pilocarpine injection (Fig. 5*A*) or the animal's latency to SE (Fig. 5*B*). Thus, pretreatment with TG6-10-1 did not alter the susceptibility to or progression of pilocarpine-induced convulsive SE. Two other possibilities are that TG6-10-1 altered (*i*) the efficacy of pentobarbital during pharmacological suppression of SE or (*ii*) the subsequent reentrance into SE during recovery from pentobarbital treatment. In the second experiment, therefore, mice were implanted with cortical wire electrodes for continuous electroencephalography (EEG) recording to determine whether TG6-10-1 altered electrographic activity during pilocarpine-induced SE. Cortical EEG recording revealed that systemic administration of TG6-10-1 after pilocarpine-induced SE did not modify the frequency or intensity of abnormal epileptiform spikes over the 48-h period after pilocarpine injection (Fig. 5 *C* and *D*). Moreover, during the first 7 d after pilocarpine SE, the frequency of spontaneous seizures was not affected by TG6-10-1 (Fig. S3). These results demonstrate that the EP2 antagonist does not affect generalized spiking or the typical reappearance of SE several hours after a single dose of pentobarbital, but do not rule out changes in focal epileptiform activity restricted to a deep brain region.

Discussion

We show that an EP2-selective competitive antagonist with 10-fold weaker potency against DP1, when administered systemically beginning 4 h after onset of pilocarpine-induced SE, mitigates many of the deleterious consequences of SE including delayed mortality, weight loss, functional deficit, opening of the blood–brain barrier, formation of a cytokine storm, gliosis, and neurodegeneration in the hippocampus. Because COX-2 produces five prostanoid products that act on nine different receptors (1), it was unexpected

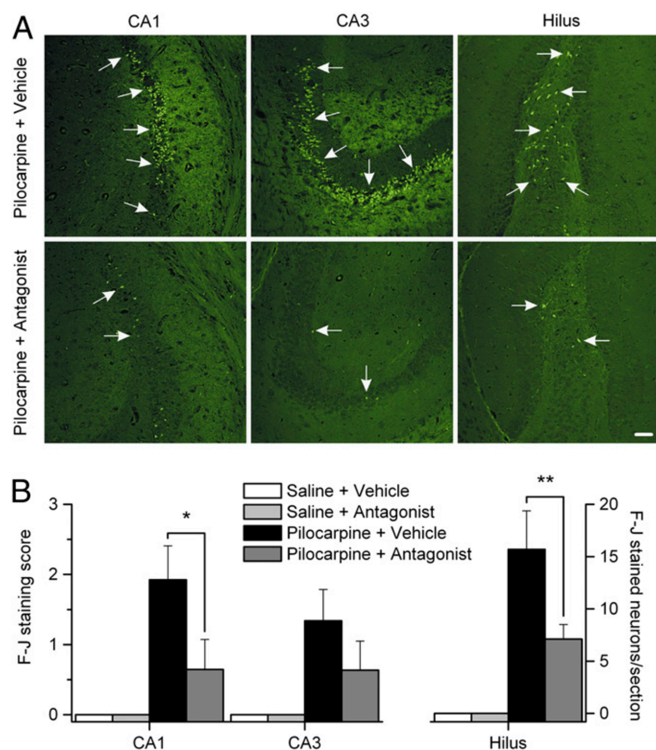

Fig. 4. EP2 receptor antagonist reduces neurodegeneration in hippocampus after SE. (*A*) Neurodegeneration in hippocampi from animals treated with vehicle or EP2 antagonist TG6-10-1 was assessed by Fluoro-Jade staining 4 d after pilocarpine-induced SE. Arrows point to damaged neurons. No positive staining was detected in control mice treated with vehicle or TG6-10-1 only (Fig. S2). (Scale bar, 50 μm.) (*B*) Quantification of neurodegenerating neurons in hippocampal subregions CA1, CA3, and dentate hilus. Coronal brain sections were examined for neurodegeneration with Fluoro-Jade (F-J) staining. One of every five sections was counted throughout the hippocampus. Neuronal injury in CA1 and CA3 was quantified by averaging the injury scores of sections from the same animal (in each section: 0, <3 Fluoro-Jade–positive cells; 1, 3–30 cells; 2, 31–100 cells; 3, extensive Fluoro-Jade staining, frequently in patches). Neuronal injury in the hilus was evaluated by counts of Fluoro-Jade–positive cells per section ($n = 8$–9 mice per group, $^*P < 0.05$, $^{**}P < 0.01$, one-way ANOVA and post hoc Bonferroni test with selected pairs). Data are shown as mean ± SEM.

that systemic inhibition of a single prostanoid receptor, EP2, would completely recapitulate the multiple beneficial effects of conditionally ablating COX-2 from a restricted population of forebrain neurons (11, 19). By contrast, the EP1 receptor appears to be responsible for much of the neuronal injury that follows cerebral ischemia (31). These results point to an important role for the EP2 receptor and perhaps DP1 in the neuropathogenesis of SE. More importantly, our finding raises the possibility that an EP2 antagonist could be used as adjunctive treatment of prolonged SE to reduce delayed mortality. Delayed mortality in patients after SE is often associated with acute or gradual cardiac decompensation (32). Pilocarpine-treated rats also develop chronic changes in autonomic control of cardiac function characterized by decreased parasympathetic activity leading to sympathetic dominance (33–35) and increased risk for ventricular arrhythmias. The location of the EP2 receptor responsible for SE-associated delayed mortality is not known, although a similar reduction in delayed mortality was observed in a conditional knockout of COX-2 limited to principal forebrain neurons (11), suggesting the relevant EP2 receptor is central rather than peripheral. Additional work is needed to test the hypothesis that EP2 receptor activation modulates cortical networks that influence brainstem circuits responsible for sympathovagal balance.

Brain inflammation is now recognized as a common feature of chronic neurodegenerative disorders, with the COX-2 cascade being widely believed to play a central role (36, 37). The role of the EP2 receptor in inflammation appears to be tissue- and context-dependent. In the periphery, genetic ablation of EP2 inhibits phorbol ester-induced expression of IL-1α and macrophage infiltration into the skin (38). In the CNS, however, selective EP2 activation reduces microglial migration toward injured tissue (39), whereas ablation of the EP2 receptor limits lipopolysaccharide-induced, microglia-mediated inducible nitric oxide synthase (iNOS) production and neurotoxicity in mixed cortical cultures (40). As the resident forms of macrophages in the brain, microglia appear to play a pivotal role in seizure-induced immune responses because activated microglia are a major source of a host of proinflammatory and neurotoxic factors including cytokines, chemokines, free radicals, and prostanoids in injured neuronal tissues (41, 42); activated astrocytes might also contribute to brain inflammation by producing iNOS in response to EP2 activation (43). Our work shows that an EP2 receptor antagonist significantly reduced the seizure-mediated induction of seven cytokines and chemokines, among them IL-1β and IL-6, and also blunted the induction of activated glial markers (Fig. 2).

IL-1β plays critical roles in a variety of cellular events such as cell differentiation, proliferation, and apoptosis, and inhibition of either IL-1β synthesis or its receptor attenuates epileptiform activity in a chronic model of epilepsy (44). Interestingly, IL-1β can induce COX-2 in the brain (45). IL-6 is one of the major mediators of both the acute phase and chronic responses during inflammation (46, 47). IL-6 can also induce COX-2 (48). Elevated COX-2 in turn synthesizes more PGE_2 to maintain EP2 receptor activation. This self-reinforcing cycle of EP2 receptor activation could contribute to long-term inflammation and sustained neuronal injury, which might underlie a molecular mechanism of chronic neuronal inflammation and injury in a range of neurodegenerative diseases. Inhibition of the EP2 receptor could break this cycle and, therefore, reduce chronic inflammatory reactions (Fig. 6). The anti-inflammatory effects of the EP2 receptor antagonist suggest that the EP2 receptor plays a significant role in immune responses in the brain, as it does in the periphery.

Disruption of the blood–brain barrier is another common event in numerous neurological disorders including seizures and stroke (26, 27, 49), and contributes to the development of brain injury. Whether blood–brain barrier disruption is only a consequence of seizures or can also affect progression of the disease has been questioned for decades (50). However, recent evidence indicates that leukocyte infiltration together with signals via a leaky blood–brain barrier and astrocyte-derived cytokines enhance inflammation in the brain and contribute to epileptogenesis (26, 28, 51), although the immune responses mediated by those infiltrated leukocytes might afford some counteracting benefits (52).

Importantly, extravasation of albumin into the brain after SE appears to promote the progression of epilepsy, because injection of albumin directly into the brain promotes gliosis and intensifies subsequent development of spontaneous seizures (29). COX-2 and prostanoid signaling pathways have long been known to be involved in regulation of blood–brain barrier permeability (53). Recently, the prostaglandin receptor EP1 has been reported to promote blood–brain barrier leakage by tyrosine phosphorylation of occludin at tight junctions after cerebral ischemia (49). In addition, during seizures, EP1 receptor activation up-regulates blood–brain barrier efflux transporter P-glycoprotein, which reduces brain access and efficacy of therapeutic agents such as phenytoin (54). In our study, leakage of serum albumin into the brain via a damaged blood–brain barrier after SE was abolished by an EP2 receptor antagonist (Fig. 3). Our results indicate a role for the prostaglandin receptor EP2 in neuroinflammation and blood–brain barrier disruption after SE. Blood–brain barrier opening and brain inflammation interact

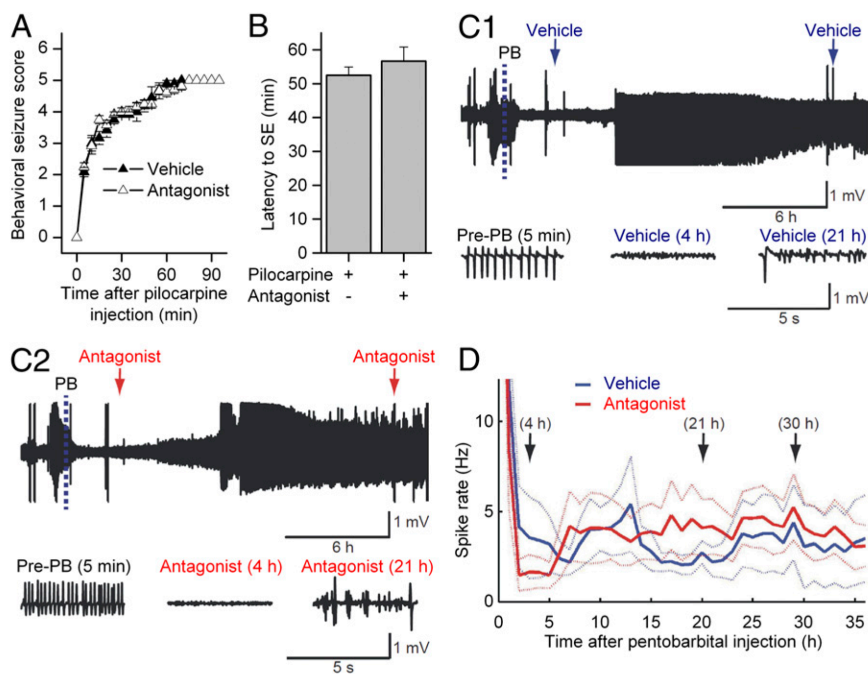

Fig. 5. EP2 receptor antagonist is not an acute anticonvulsant in the mouse pilocarpine model. (*A*) Mice were injected with vehicle or EP2 antagonist TG6-10-1 (5 mg/kg, i.p.) and 1 h later with pilocarpine (280 mg/kg, i.p.). The behavioral seizure score was tabulated every 5 min (n = 12 mice per group). (*B*) The latency to reach behavioral SE after pilocarpine injection. (*C*) Representative cortical EEG recordings from (*C1*) vehicle- and (*C2*) TG6-10-1–treated mice. The dashed line indicates the time of administration of pentobarbital (PB), whereas the arrows denote the time of drug (5 mg/kg, i.p.) or vehicle administration. (*Lower*) Traces are temporally expanded portions of the EEG recording either before administration of pentobarbital (5 min) or at the time of administration of either vehicle or TG6-10-1 (4, 21 h). Seizure activity was quantified by the analysis of the rate of EEG spiking. (*D*) Comparison of spike rate [mean (solid lines) ± 95% confidence intervals (CIs, dashed lines)] showed overlapping 95% CIs for EEG spike rate between vehicle- and TG6-10-1–treated animals at every time point (n = 14 mice per group). The arrows indicate the time of administration of drug or vehicle at 4, 21, and 30 h after SE onset.

with and enhance each other, with the blood–brain barrier usually becoming more permeable during brain inflammation. Taken together, activation of both prostaglandin receptors EP1 and EP2 are involved in blood–brain barrier breakdown. EP1 receptor activation directly modifies blood–brain barrier components, whereas the mechanism by which the EP2 receptor mediates blood–brain barrier breakdown, and the cellular targets, is not yet known.

Although downstream COX-2 signaling pathways promoting neurodegeneration are not completely understood, recent evidence suggests that EP2 receptor activation by PGE_2 might underlie neuronal injury in some models of chronic inflammation and neurodegeneration (1, 4–6). Global ablation of the EP2 receptor reduced oxidative stress and improved survival in animal models of Alzheimer's disease and amyotrophic lateral sclerosis (5, 6). We demonstrate here that an EP2 receptor antagonist significantly decreases delayed neurodegeneration in mice when administered after SE (Fig. 4 and Fig. S2). By contrast, EP2 ablation can *increase* infarct volume in models of focal ischemia (55), and we have shown that intraventricular administration of an EP2 agonist immediately after SE can be neuroprotective in a rat pilocarpine model (19). These seemingly incongruent observations are probably due to the complexity of inflammatory signaling in the brain, and could reflect dual consequences of EP2 activation—early neuroprotection followed by later neurodegeneration.

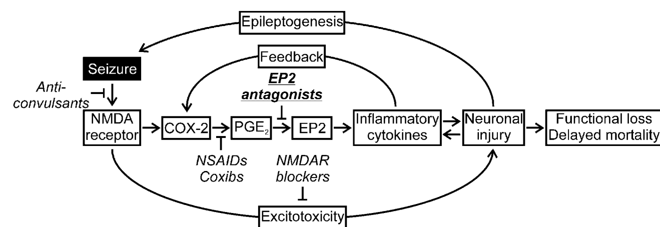

Fig. 6. Proposed model for the inflammatory action of EP2 signaling following SE. SE-induced COX-2 increases brain PGE_2 level. PGE_2 signaling through the EP2 receptor up-regulates inflammatory cytokines including IL-1β, IL-6, and TNF-α, which further induce COX-2 expression to maintain PGE_2/EP2 signaling. This positive feedback loop might contribute to chronic inflammation following SE. Nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2–selective inhibitors (Coxibs) can reduce PGE_2 levels, but also levels of other prostanoids that may have beneficial functions.

Continuous electrographic recordings for 48 h showed that TG6-10-1 is not a frank anticonvulsant in the pilocarpine model; furthermore, TG6-10-1 had no detectable effect on pilocarpine-induced electrographic activity, thus confirming a different mode of action from benzodiazepines, phenytoin, propofol, or pentobarbital. Neuroprotection by an EP2 receptor antagonist supports the involvement of this key prostaglandin receptor in delayed neurodegeneration after SE. The electrographic data support the hypothesis that neuroprotection by EP2 inhibition after SE is *not* the consequence of a direct anticonvulsant effect (Fig. 5 *C* and *D* and Fig. S3); rather, the neuroprotective effect of TG6-10-1 likely derives from the anti-inflammatory actions of the compound (Fig. 6). This conclusion is supported by extensive evidence concerning the neuroprotective mechanisms of IL-1β inhibitors (56), TNF-α inhibitors (57), and COX-2 inhibitors (14–18), because the targets of these inhibitors are down-regulated by the EP2 antagonist (Fig. 2*A*). The cellular targets of the EP2 antagonist, the mechanism by which blood–brain barrier breakdown is prevented by the EP2 antagonist, and the role—if any—of the DP1 receptor in these phenomena are all important topics for future study. Nonetheless, our findings reinforce the notion that the prostaglandin receptor EP2 should be explored as a therapeutic target to oppose neuroinflammation and neurodegeneration, recognizing the cardio- and cerebrovascular adverse effects during long-term use of selective COX-2 inhibitors (21). Our results also support the notion of EP2 antagonism as an adjunctive strategy, along with benzodiazepines and general anesthetics, to treat status epilepticus.

Materials and Methods

Please see *SI Materials and Methods* for details about cell culture, chemicals and drugs, cell-based cAMP assay, potency of TG6-10-1 on prostanoid receptors, off-target activity, pharmacokinetics, animals and seizure model, quantitative real-time PCR, Western blot, histopathology, and EEG analysis.

Drug Administration After Pilocarpine Treatment. Mice underwent SE for 1 h, and SE was then terminated by pentobarbital (30 mg/kg in saline, i.p.). After 3 h, mice were randomized and received three doses of vehicle (10% DMSO,

50% PEG 400, 40% ddH_2O) or TG6-10-1 (5 mg/kg, i.p.) at 4, 21, and 30 h after SE onset. Mice were fed moistened rodent chow, monitored daily, and injected with 5% (wt/vol) dextrose in lactated Ringer's solution (Baxter) (0.5 mL, s.c.) when necessary. Four days after SE, a group of mice was euthanized under deep isoflurane anesthesia and perfused with PBS to wash blood out of the brain, and then brains were collected. The hippocampus and cortex from one cerebral hemisphere were dissected and stored at −80 °C for further use; the other cerebral hemisphere was immersed in 4% paraformaldehyde fixative for at least 10 h for histology study. All experiments were approved by the Institutional Animal Care and Use Committee of Emory University and conducted in accordance with its guidelines. Every effort was made to minimize animal suffering.

Statistical Analysis. Statistical analyses were performed using Prism (GraphPad Software) by one- or two-way ANOVA with post hoc Bonferroni test, Fisher's exact test, or *t* test as appropriate. Survival was assessed using Kaplan–Meier analysis. $P < 0.05$ was considered to be statistically significant. All data are presented as mean $\pm$ SEM.

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