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The prostaglandin EP1 receptor potentiates kainate receptor activation via a protein kinase C pathway and exacerbates status epilepticus

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ABSTRACT

Prostaglandin E2 (PGE2) regulates membrane excitability, synaptic transmission, plasticity, and neuronal survival. The consequences of PGE2 release following seizures has been the subject of much study. Here we demonstrate that the prostaglandin E2 receptor 1 (EP1, or *Ptger1*) modulates native kainate receptors, a family of ionotropic glutamate receptors widely expressed throughout the central nervous system. Global ablation of the EP1 gene in mice (EP1-KO) had no effect on seizure threshold after kainate injection but reduced the likelihood to enter status epilepticus. EP1-KO mice that did experience typical status epilepticus had reduced hippocampal neurodegeneration and a blunted inflammatory response. Further studies with native prostanoid and kainate receptors in cultured cortical neurons, as well as with recombinant prostanoid and kainate receptors expressed in *Xenopus* oocytes, demonstrated that EP1 receptor activation potentiates heteromeric but not homomeric kainate receptors via a second messenger cascade involving phospholipase C, calcium and protein kinase C. Three critical GluK5 C-terminal serines underlie the potentiation of the GluK2/GluK5 receptor by EP1 activation. Taken together, these results indicate that EP1 receptor activation during seizures, through a protein kinase C pathway, increases the probability of kainic acid induced status epilepticus, and independently promotes hippocampal neurodegeneration and a broad inflammatory response.

Introduction

PGE2, a major cyclooxygenase 2 product in the mammalian brain, exerts hormone-like properties that modulate many physiological and pathophysiological functions, among them membrane excitability and synaptic transmission in CA1 pyramidal neurons (Chen and Bazan, 2005). However, the pathways and mechanisms involved remain largely unknown. Kainic acid, an excitatory neurotoxin, when injected into rodents at doses ≥20 mg/kg induces seizures that can progress into status epilepticus, which in turn eventually causes development of spontaneous recurrent seizures (epilepsy) in the weeks following (Ben-Ari et al., 1979; Hellier et al., 1998). Kainate receptors (KARs) are ionotropic glutamate receptors composed of GluK1 through GluK5 subunits that are located both presynaptically and postsynaptically throughout the CNS and are involved in synaptic plasticity and transmission (Huettner, 2003; Kamiya, 2002; Lerma, 2003; Pinheiro and Mulle, 2006). Recently we demonstrated expression of the high affinity kainate receptor subunits (GluK4 and GluK5) in the CA3 region of the hippocampus (Rojas et al., 2013), which supported a previous report by Darstein et al. (2003). The expression profile of the high affinity kainate receptor subunits is consistent with the localization of kainic acid binding in the hippocampus. Furthermore, the expression profile of GluK5 (one of the high affinity KA subunits) correlates with the neurodegeneration pattern in the hippocampus following kainic acid injection in rodents.

A prominent neuropathology associated with kainic acid induced status epilepticus is hippocampal neurodegeneration. Recent studies have suggested that signaling via the prostaglandin EP1 receptor may affect the fate of neurons following brain injury. For example, EP1 deficient mice show less neuronal injury following transient forebrain ischemia (Shimamura et al., 2013) and cerebral ischemia (Zhen et al., 2012). Pharmacological inhibition of the EP1 receptor with SC51089 reduces neuronal loss and blood–brain barrier disruption following ischemic injury (Fukumoto et al., 2010; Shimamura et al., 2013) suggesting that EP1 activation may promote cell death. Kawano et al. (2006) demonstrated that EP1 gene inactivation reduced brain injury following NMDA induced excitotoxicity, ischemia or oxygen glucose deprivation,

Abbreviations: GluK, kainate receptor; PGE2, prostaglandin E2; $PGF2_{\alpha}$, prostaglandin F2α; EP1, prostaglandin E2 receptor 1; EP2, prostaglandin E2 receptor 2; EP1-KO, prostaglandin E2 receptor 1 knockout mice; WT, C57Bl/6 wildtype mice; EC_{50}, half maximal effective concentration; ED_{50}, effective dose for 50% of subjects; PKC, protein kinase C; PKA, protein kinase A; HBSS, Hank's balanced salt solution; ANOVA, analysis of variance; KAR, kainate receptor; KA, kainic acid; Dom, domoic acid; SE, status epilepticus; GFAP, Glial fibrillary acidic protein; MAP2, microtubule-associated protein 2; Iba1, ionized calcium-binding adapter molecule 1; CA1, *Cornu Ammonis* 1; CA3, *Cornu Ammonis* 3; CT, cycle threshold; Con, control.

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suggesting that the presence of EP1 in normal animals contributes to or exacerbates the injury. Each glutamate receptor subtype (NMDA, AMPA and KA) is likely to play a role in the above mentioned brain injury models. Endogenous kainate receptors are regulated by $G_{\alpha q}$ coupled receptors that are known to modulate excitotoxicity following seizures (Benveniste et al., 2010; Rojas et al., 2013). EP1 is a $G_{\alpha q}$-coupled receptor for PGE2, thus we hypothesized that kainate receptors are targeted by EP1 pathways to contribute to the neuropathology that follows status epilepticus. Here we ask the questions: Does genetic inactivation of EP1 alter kainate induced status epilepticus? Do EP1 knockout mice display reduced neurodegeneration or brain inflammation following kainate induced status epilepticus? Is there cross-talk between kainate receptors and prostanoid receptors and if so, what is the mechanism? To address these questions we combined an in vivo rodent model of kainate induced status epilepticus and functional in vitro studies of native and co-expressed recombinant kainate receptors and prostanoid receptors.

Materials and methods

Kainic acid injection

All procedures and experiments conformed to the guidelines of the Animal Care and Use Committee of Emory University. Every effort was made to minimize animal suffering. Wildtype (WT) adult male C57BL/6 mice (≥20 g) were obtained from Charles Rivers Labs (Wilmington, MA, USA). EP1 knockout mice (EP1-KO) (Ptger1^{tm1Dgen}; stock number 011638) were purchased from the Mutant Mouse Regional Resources Center (MMRRC) through the Jackson Laboratory. Disruption of the EP1 gene had been produced by targeted insertion of the LacZ gene that drives β-galactosidase activity. The EP1-KO mice display reduced blood pressure and impulsive behavior (Guan et al., 2007; Matsuoka et al., 2005; Stock et al., 2001). Otherwise, they appear normal and their brains develop normally. The Jackson Laboratory C57BL/6 mice show a higher mortality than the Charles Rivers Laboratories C57BL/6 mice during status epilepticus (Borges et al., 2003) and thus the EP1-KO mice were bred for six to eight generations from the C57BL/6 Jackson Laboratory strain into the C57BL/6 Charles River Laboratories strain to generate homozygous knockouts. Mice were housed under a 12 hour light/dark cycle with food and water ad libidum. Total RNA was isolated from half brains of wildtype and EP1-KO mice injected with kainate four days prior to verify disruption of the EP1 gene by a targeted insert of the LacZ gene. The presence of a 584 bp amplicon that represents the LacZ targeted gene insertion used to disrupt the EP1 gene suggests functional EP1 disruption in the 14 brains taken from EP1-KO mice that experienced status epilepticus. End point PCR was performed on the samples using two sets of primers; one set to identify wildtype (a 272 bp amplicon) and the other set to identify the EP1 disrupted gene (a 584 bp amplicon). The primer sequences are as follows: wildtype forward: 5CCAACAGGCGATAATGGCACATCAC-3′; EP1-KO (target insertion) forward: 5′-GGGGATCGATCCGTCCTGTAAGTCT-3′; and a common reverse: 5′-ACCATGCAGCCACCCAGGAAATGAC-3′ for both. The PCR products were separated on a 1% ethidium bromide agarose gel and imaged under UV. In a separate experiment total RNA was also isolated from the hippocampi of 8 untreated male wild-type C57BL/6 mice to verify expression of the EP1 gene. End point PCR was performed using the wildtype primer set as described above. We could not use immunohistochemistry to verify loss of the EP1 receptor because three different antibodies showed the same pattern of hippocampal pyramidal cell labeling in sections obtained from wildtype mice and EP1-KO mice prepared by two different strategies.

Kainate was obtained from Tocris Bioscience (Ellisville, MO) and was dissolved at 4 mg/ml in a physiological (0.9%) saline (pH 7.4) to generate a stock solution. The stock solution was diluted in 0.9% saline to create fresh working solutions on the injection day. Mice were weighed and injected with a single dose of kainate (5, 10, 20, 30 or 40 mg/kg) subcutaneously (s.c.) at 10 ml/kg. Subcutaneous injection was chosen as the route of administration for the single dose exposure to eliminate any complications from the injection (e.g. injecting into an internal organ). Control mice received 0.9% saline instead of kainate. Additional groups of wildtype and EP1-KO mice were injected with only a high dose of kainic acid (30 or 40 mg/kg) intraperitoneally (i.p.) to obtain a high enough proportion of mice who survive after status epilepticus; if the mice did not enter status epilepticus within 30 min they received another dose of 10 mg/kg. In mice, kainate-induced seizures consisted of distinct motor behaviors, including forelimb clonus, loss of posture, rearing, and falling. Animals presenting these behaviors with increased seizure intensity, duration, and frequency shortly after the injection of kainate were declared to be in status epilepticus, which is characterized in the kainate model by periodic rearing and falling accompanied by whole body clonic seizures. Behavior was scored using a modified Racine scale (Racine, 1972) shown below. All mice that entered status epilepticus continued seizing for at least 90 minutes; seizures usually persisted for several hours and eventually waned and stopped. To increase survival of animals, hyperthermia was minimized during status epilepticus by periodic cooling of the animals with chilled air. Following status epilepticus the mice were hydrated with lactate ringers and allowed to recover overnight.

Behavioral score		Observed motor behavior
0	Normal behavior	Walking, exploring, sniffing, grooming
1	Freeze behavior	Immobile, staring, heightened startle, curled-up posture
2	Automatisms	Blinking, head bobbing, scratching, face washing, whisker twitching, chewing, star gazing
3	Early seizure behavior	Myoclonic jerks, partial or whole body clonus (flexions and extensions of body muscles)
4	Advance seizure behavior	Rearing and falling, loss of posture (e.g. falling, corkscrew turning, splaying of limbs)
5.	Status epilepticus	Repeated seizure activity (≥2 events in stages 3, 4 or 6 within a 5 minute window), which lasts ≥30 min
6	Intense seizure behavior	Repetitive jumping or bouncing, wild running, tonic seizures
7	Death	

A behavior score was assigned to an animal when it exhibited at least two different signs within that score group during a 5 min observation period.

FluoroJade labeling

Four days after status epilepticus onset, wildtype and EP1-KO mice along with the saline controls were anesthetized deeply with isoflurane. The mice were decapitated and their brains were rapidly removed and longitudinally bisected. One-half of the brain was fixed overnight in a 4% paraformaldehyde solution at 4 °C. The other half excluding the cerebellum and hind brain was frozen on dry ice and kept for RNA isolation. The next day the half brains post-fixed in 4% paraformaldehyde were transferred to 30% (w/v) sucrose in phosphate buffered saline at 4 °C until they sank. Fixed mouse half brains were dehydrated, embedded in paraffin and sectioned (8 μm) coronally through the hippocampus and mounted onto slides. Every 20th hippocampal section was labeled with cresyl violet. Every 5th section excluding those used for cresyl violet for a total of ~40 sections from each half mouse brain was used for FluoroJade staining to label degenerating cells according to the manufacturer protocol (Histo-chem Inc., Jefferson, AR) as described by Schmued et al. (1997). Briefly, slides were immersed in 100% ethyl alcohol for 3 min followed by a 1 min immersion in 70% alcohol and a 1 min immersion in distilled water. The slides were then transferred to a solution of 0.06% potassium permanganate for 15 min and were gently shaken on a rotating platform at 25 °C. The sections were rinsed for 1 min in distilled water and then transferred to the 0.001% FluoroJade B staining solution where they were gently agitated for 30 min.