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Research report

Altered GABAergic neurotransmission is associated with increased kainate-induced seizure in prostaglandin-endoperoxide synthase-2 deficient mice

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Abstract

Excitotoxicity involves over activation of brain excitatory glutamate receptors and has been implicated in neurological, neurodegenerative and neuropsychiatric diseases. Metabolism of arachidonic acid (AA) through the phospholipase A_2 (PLA_2)/prostaglandin-endoperoxide synthase (PTGS) pathway is increased after excitotoxic stimulation. However, the individual roles of the PTGS isoforms in this process are not well established. We assessed the role of the PTGS isoforms in the process of excitotoxicity by exposing mice deficient in either PTGS-1 (PTGS-$1^{-/-}$) or PTGS-2 (PTGS-$2^{-/-}$) to the prototypic excitotoxin, kainic acid (KA). Seizure intensity and neuronal damage were significantly elevated in KA-exposed PTGS-$2^{-/-}$, but not in PTGS-$1^{-/-}$, mice. The increased susceptibility was not associated with an alteration in KA receptor binding activity or mediated through the CB1 endocannabinoid receptor. The frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) was decreased in the CA1 pyramidal neurons of PTGS-$2^{-/-}$ mice, suggesting an alteration of GABAergic function. In wild-type mice, six weeks treatment with the PTGS-2 selective inhibitor celecoxib recapitulated the increased susceptibility to KA-induced excitotoxicity observed in PTGS-$2^{-/-}$ mice, further supporting the role of PTGS-2 in the excitotoxic process. The increased susceptibility to KA was also associated with decreased brain levels of PGE_2, a biomarker of PTGS-2 activity. Our results suggest that PTGS-2 activity and its specific products may modulate neuronal excitability by affecting GABAergic neurotransmission. Further, inhibition of PTGS-2, but not PTGS-1, may increase the susceptibility to seizures.

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1. Introduction

The prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase (COX), isoforms, PTGS-1 and PTGS-2, are both expressed under basal conditions in the brain [25,67]. These enzymes catalyze the first step in the transformation of arachidonic acid (AA) to prostaglandins and thromboxanes [60], bioactive metabolites of AA known to mediate physiological and pathological processes in the brain [23,24]. Release and metabolism of AA through the phospholipase A_2 (PLA_2)/PTGS pathway is increased during excitotoxicity [12], a process that involves the over activation of brain excitatory neurotransmission. Kainic acid (KA), the prototypic excitotoxin, binds to the KA receptors in the brain and induces seizures that result in inflammation, oxidative damage and neuronal death, processes that have been implicated in neurological, neurodegenerative and psychiatric diseases [16,20,40,49,52,66,68,71,72]. Although it has been demonstrated that PTGS plays a role in excitotoxicity, the distinct role of the two PTGS isoforms has not been fully established.

Previous attempts to study the role of PTGS isoforms in the process of excitotoxicity by using non steroidal

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anti-inflammatory drugs (NSAIDs), pharmacological inhibitors of PTGS, have not fully characterized specific roles of each isoform. However, a distinct dichotomy in the effect of gross PTGS inhibition on the process of excitotoxicity has been suggested, with both protective and detrimental effects of PTGS inhibition being demonstrated in studies using pharmacological inhibitors [4,34]. While these studies have examined the effects of acute inhibition of PTGS before or after excitotoxic stimuli [5,9,18,33,34,61], the consequences of long-term inhibition of PTGS activity on the process of excitotoxicity remain to be addressed. A thorough understanding of these consequences is clinically relevant since a common therapeutic approach to treating pain and inflammation involves long-term treatment with NSAIDs.

In order to clarify the specific roles of PTGS-1 and PTGS-2 in the process of KA-induced excitotoxicity and to determine the effect of long-term PTGS inhibition on this process, we employed a novel approach using mice deficient in either PTGS-1 (PTGS-$1^{-/-}$) or PTGS-2 (PTGS-$2^{-/-}$). We demonstrate for the first time that PTGS-$2^{-/-}$ mice, but not PTGS-$1^{-/-}$ mice, are more susceptible then wild type mice to systemically-injected, KA-induced seizures and neuronal damage. Further, we confirm our observation by demonstrating that mice pretreated for six weeks with celecoxib, a PTGS-2 specific inhibitor, also have augmented susceptibility to KA-induced excitotoxicity. Whole cell recording in hippocampal slices and measurement of brain prostaglandin levels revealed alterations in PTGS-$2^{-/-}$ mouse neurophysiology and neurochemistry that may contribute to the increased susceptibility to KA-induced excitotoxicity. Specifically, the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) was decreased in the CA1 pyramidal neurons of PTGS-$2^{-/-}$ mice, suggesting an alteration of GABAergic function. Augmented susceptibility to excitotoxicity was not mediated by endocannabinoid CB1 receptor signaling due to AA metabolites catabolized by PTGS-2, but not PTGS-1 [30–32,70], that are known to alter neuronal excitability [3,28].

2. Methods and materials

2.1. Animal housing

All procedures were performed under an animal protocol approved by the NICHD ACUC in accordance with NIH guidelines on the care and use of laboratory animals. Male COX-$1^{-/-}$, COX-$1^{+/+}$, COX-$2^{-/-}$, COX-$2^{+/-}$, and COX-$2^{+/+}$ mice maintained on a C57Bl/6-129/Ola background for over 35 generations [35,41] were received at our animal facility at 6 weeks of age from a private NIEHS colony maintained by Taconic Farms (Germantown, NY) separately from their commercially available colony. In order to prevent the inclusion of strain or genetic background confounders between COX deficient and wild type mice, all of the mice used in this study were derived from a maintenance colony of COX-$1^{+/-}$ or COX-$2^{+/-}$ males and females, as previously described [35,41,64]. Specifically, all mice used in this study were progeny derived from heterozygous by heterozygous matings and therefore all contained the same strain and genetic background. This is an important point to recognize since genetic background and mouse strain has been demonstrated to alter the susceptibility to KA-induced excitotoxicity [38,39,54,55]. For celecoxib pretreatment, PTGS-$2^{+/+}$ mice were given free access for 6 weeks to a diet containing 0, 3000 or 6000 ppm celecoxib. Celecoxib is a COX-2 specific inhibitor with a selectivity ratio of >375 [13]. Celebrex™ capsules (400 mg; Pfizer Inc., New York, NY) were obtained from the NIH Division of Veterinary Medicine and were incorporated into feed by Research Diets, Inc. (New Brunswick, NJ) [73].

2.2. Kainate injections and seizure observation

Mice (males, 12–14-week-old) were injected intraperitoneally (i.p., injection volume 100–300 μl) with 10 mg/kg KA (Biomol International, Plymouth Meeting, PA; 2 mg/ml in 0.9% saline) or vehicle and then videotaped for 2 h after injection. A dose–response pilot study (data not shown) ranging from 10–25 mg/kg determined that 10 mg/kg KA dissolved in 0.9% saline caused seizures but did not result in fatalities in wild type mice. Seizure intensity was rated using a modified Racine seizure scale [48,55]: 0 = no behavioral alteration; 1 = immobility, mouth and facial movements, facial clonus; 2 = head nodding, forelimb and/or tail extension, rigid posture; 3 = forelimb clonus, repetitive movements; 4 = rearing, forelimb clonus with rearing, rearing and falling; 5 = continuous rearing and falling, jumping; 6 = severe tonic–clonic seizures. Mice were assigned a Racine seizure score (RSS) for each 5 min interval over the course of the 2 h session, after which a median RSS was calculated for the entire 2 h session for each mouse.

2.3. Pretreatment with the CB1 receptor antagonist AM-251

Thirty minutes before KA injection, mice were injected i.p. with 30 mg/kg of the CB1 antagonist, AM-251(Tocris Bioscience, Ellisville, MO), dissolved in DMSO, Tween-80, ethanol and 0.9% saline or vehicle as previously described [2,59]. AM-251 peak brain concentrations have previously been observed at 30 min post injection and 30 mg/kg is a dosage at which a plateau in brain to plasma ratio is reached [17,59].

2.4. Histochemistry and autoradiography

Sagittal brain sections from mice euthanized 24 h after KA injection were cut on a cryostat (Bright Instrument Company, Ltd.; Huntingdon, England). Cresyl violet staining was performed as described [46]. FJB (Histo-Chem, Inc., Jefferson, AR) staining was performed as described previously using postfixed tissue samples [56]. FJB and cresyl violet staining were quantified using an ordinal pathology index [6]; 0 = no pathology; 1 = mild pathology; 2 = moderate pathology; 3 = severe pathology.

[^{3}H] KA receptor autoradiography was performed as previously described [22,43]. Slides were apposed to Biomax MR Film (Kodak, Rochester, NY) for 6–8 weeks and images were analyzed using NIH Image. Optical density for specific brain regions was transformed to tissue equivalent by using autoradiographic microscales (GE Healthcare Life Sciences, Piscataway, NJ).

2.5. Measurement of plasma celecoxib levels by HPLC

Plasma was collected by cardiac puncture (Vacutainer PST gel and lithium heparin tube; Becton-Dickenson, Franklin Lakes, NJ) from the cage mates of mice used in KA experiments. Celecoxib concentration was determined by HPLC with UV detection as previously described [74].

2.6. Determination of brain prostaglandin concentration by ELISA

KA-naïve mice pretreated with celecoxib were euthanized by head-focused microwave fixation (90% power, 0.9 s; Cober Electronics, Stamford, CT). Brains were prepared and analyzed for prostaglandin measurement as previously described [7].

2.7. Whole cell recordings of CA1 pyramidal neurons

Slice preparation and whole cell recording were performed as previously described [44,65]. Mice (3–4 weeks of age) brains were placed in an ice-cold slicing solution containing: 85 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 4 mM $MgCl_2$, 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 25 mM glucose, 75 mM sucrose, pH 7.4;