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Soluble epoxide hydrolase activity regulates inflammatory responses and seizure generation in two mouse models of temporal lobe epilepsy

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ABSTRACT

Neuroinflammation is known to be involved in epileptogenesis with unclear mechanisms. Inhibition of soluble epoxide hydrolase (sEH) seems to offer anti-inflammatory protection to ischemic brain injury in rodents. Thus, it is hypothesized that sEH inhibition might also affect the neuroinflammatory responses caused by epileptic seizures. In the present study, we investigated the involvement of sEH in neuroinflammation, seizure generation and subsequent epileptogenesis using two mouse models of temporal lobe epilepsy. Experimental epileptic seizures were induced by either pilocarpine or electrical amygdala kindling in both wild-type (WT) C57BL/6 mice and sEH knockout (sEH KO) mice. The sEH expression in the hippocampus was detected by immunohistochemistry and Western blot analysis. The effects of the sEH hydrolase inhibitors, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA) and N-[1-(1-oxopropyl)-4-piperidinyl]-N′-[4-(trifluoromethoxy) phenyl)-urea (TPPU), and of the genetic deletion of sEH on seizure-induced neuroinflammatory responses and the development of epilepsy were evaluated. In the hippocampus of WT mice, sEH was mainly expressed in astrocytes ($GFAP^+$), neurons ($NeuN^+$) and scattered microglia ($Iba\text{-}1^+$) in the regions of CA1, CA3 and dentate gyrus. Expression of sEH was significantly increased on day 7, 14, 21 and 28 after pilocarpine-induced status epilepticus (SE). Administration with sEH inhibitors attenuated the SE-induced up-regulation of interleukin-1β (IL-1β) and interleukin-6 (IL-6), the degradation of EETs, as well as IκB phosphorylation. Following treatment with AUDA, the frequency and duration of spontaneous motor seizures in the pilocarpine-SE mice were decreased and the seizure-induction threshold of the fully kindled mice was increased. Up-regulation of hippocampal IL-1β and IL-6 was found in both WT and sEH KO mice after successful induction of SE. Notably, sEH KO mice were more susceptible to seizures than WT mice. Seizure related neuroinflammation and ictogenesis were attenuated by pharmacological inhibition of sEH enzymatic activity but not by sEH genetic deletion. Therefore, sEH may play an important role in the generation of epilepsy. Furthermore, the effectiveness of AUDA in terms of anti-inflammatory and anti-ictogenesis properties suggests that it may have clinical therapeutic implication for epilepsy in the future, particularly when treating temporal lobe epilepsy.

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1. Introduction

Temporal lobe epilepsy is the most common type of focal epilepsy, and is characterized clinically by spontaneous recurrent seizures (SRS) and hippocampal sclerosis. In humans and experimental animals, hippocampal sclerosis is characterized by tissue shrinkage, selective loss of neurons in CA1, CA3 and dentate hilus as well as reactive gliosis (Buckmaster, 2004; Silva et al., 2002). Several studies have proposed that a neuroinflammatory response is a critical event in these structural and functional changes that affect the hippocampal formations (Jankowsky and Patterson, 2001) and may play a role in epileptogenesis (Buckmaster et al., 2002; Dalby and Mody, 2001).

During neuroinflammation, the pro-inflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which are produced by activated microglia or astrocytes (Aisen and Davis, 1994; Hauss-Wegrzyniak et al., 1998; Vezzani et al., 2013; Xanthos and Sandkuhler, 2014), provoke pathological signaling cascades via the activation of phospholipase C (Lee et al., 2003) and phospholipase A2 (Balsinde et al., 2002; Rosenberger et al., 2004). Subsequently, non-esterified arachidonic acid (AA) is released from phospholipids and lysophospholipids and bioactive eicosanoids are formed by oxidizing enzymes (Balsinde et al., 2002; Shimizu and Wolfe, 1990). Three enzyme systems, cyclooxygenases, lipoxygenases and cytochrome P450 (CYP) epoxygenases metabolize the released AA converting them into lipid metabolites, namely the prostaglandins, leukotrienes, and epoxyeicosatrienoic acids (EETs), respectively (Phillis et al., 2006). Brain parenchymal tissue metabolizes AA via the CYP epoxygenase to EETs, which regulate cerebral blood flow (Iliff et al., 2007; Zhang et al., 2008) and have anti-inflammatory and anti-apoptotic effects (Spiecker and Liao, 2005; Yang et al., 2007). Recent studies with hypoxia and ischemic preconditioning experiments have shown that the increased expression of CYP epoxygenases and EETs in brain may confer protection against ischemic stroke induced by middle cerebral artery occlusion in the animal models (Alkayed et al., 2002; Iliff et al., 2010; Phillis et al., 2006; Simpkins et al., 2009; Zhang et al., 2007). This also suggests that EETs signaling may suppress ischemia-evoked neuroinflammation in the brain, supporting an anti-neuroinflammatory role for EETs in the brain circulation (Koerner et al., 2008).

Soluble epoxide hydrolase (sEH) is a key enzyme in the metabolic conversion of EETs into their less active form, dihydroxyeicosatrienoic acids (DHET). Therefore, inhibition of sEH is known to increase systemic levels of EETs and ameliorate the vascular and neural injury induced by cerebral ischemia (Simpkins et al., 2009; Zhang et al., 2007, 2008). However, little is known about whether the EETs-sEH pathway is involved in the pathogenesis of epilepsy.

To address this issue, the present study aimed to examine the effects of pharmacological and genetic inhibition of sEH on seizure-induced local neuroinflammation, seizure generation and epilepsy development.

2. Materials and methods

2.1. Animals, randomization and blinding procedures

All animal experiments were approved by the Institutional Animal Care and Utilization Committee (IACUC) of National Yang-Ming University, Taipei, Taiwan (approval ID: 990401), and animal care was performed in compliance with the guidelines of IACUC and the United States National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. B6.129X-*Ephx2*^{tm1Gonz}/J (sEH$^{-/-}$) mice in a C57BL/6J background were purchased from Jackson Laboratory (Bar Harbor, ME, USA). The sEH knockout (sEH KO) mice were backcrossed and bred in the Laboratory Animal Center, National Yang-Ming University, Taipei, Taiwan. Six- to eight-week-old male C56BL/6 wild type (WT) mice were ordered from BioLASCO (Taipei, Taiwan) as controls. Mice were fed a chow diet and kept on a 12-h light/dark cycle at 25 ± 2 °C in the animal room of the Laboratory Animal Center, National Yang-Ming University, Taipei, Taiwan. Animals were randomly assigned to treatment groups and numbered in random order. The experimenters were blind to the experimental treatment conditions. All further postmortem examinations were performed blindly with pseudonymization.

2.2. Animal models of epilepsy

Two models were established in this study. The pilocarpine-induced status epilepticus (SE) model was set up according to previously described procedures (Hung et al., 2013, 2012). Male mice were first pretreated intraperitoneally (i.p.) with scopolamine methylnitrate (1 mg/kg; Sigma–Aldrich, St. Louis, MO, USA) 30 m minutes before the injection of pilocarpine hydrochloride (WT mice: 325 mg/kg, i.p.; sEH KO mice: 295 mg/kg, i.p.; Sigma–Aldrich), while the age and sex matched control mice were injected with an equal volume of normal saline instead of pilocarpine. Animal behavior was monitored and only the mice exhibiting convulsive SE at stage 5 of the Racine's scale (Racine, 1972) were enrolled for subsequent experimental manipulation (Overstreet-Wadiche et al., 2006). At two hours after SE onset, the mice were treated with diazepam (10 mg/kg, i.p.; China Chemical & Pharmaceutical Co., Ltd., Taipei, Taiwan) to terminate the seizures.

The basolateral amygdala (BLA) kindling epileptogenesis model was set up according to previously described procedures (He et al., 2004). Initially, the sterile surgical implantation of electrodes was performed. Male mice were deeply anesthetized with 2–3% isoflurane inhalation (AErrane®, Baxter, Guayama, PR, USA) and placed in a Stoelting stereotaxic apparatus (51600 Lab Standard™, Stoelting Co., Wood Dale, IL, USA). Craniotomy was performed and a stimulation-recording signal electrode consisting of four Teflon-insulated 50 μm stainless steel microwires was surgically implanted into the left amygdala at coordinates of 1.0 mm posterior to bregma, 2.9 mm lateral to midline, and 4.6 mm below the dura. A stainless steel screw was implanted into the skull above the right frontal cortex as a reference electrode, and three stainless steel screws were attached to the skull to help anchor the dental cement.

After the surgical implantation of the electrodes, the kindling epileptogenesis was induced by daily electrical stimulations of BLA (He et al., 2004). After a postoperative recovery period of at least 1 week, animals were connected to an eight-channel connector containing headstage via a commutator (Plexon, Inc., Dallas, TX, USA) that permitted unrestricted movement during recording. The electrographic seizure threshold (EST) was first determined by application of 1 s train of 1 ms biphasic rectangular pulses at 60 Hz beginning at 60 μA. Additional stimulations increasing by 20 μA were administered at 1 min intervals until an electroencephalographic (EEG) seizure activity (amplitude > 2× baseline, frequency > 5 Hz, duration > 10 s) was detected. Subsequently, the experimental animals were stimulated twice daily at the stimulus intensity of the EST, with an interstimulus interval of at least 4 h. Both EEG and seizure behaviors were recorded. The seizure behaviors were classified according to the five stages described by Racine (Racine, 1972), including mouth and facial movements (stage 1), head nodding (stage 2), forelimb clonus (stage 3), seizure characterized by rearing (stage 4), and seizures characterized by rearing and falling (stage 5) (He et al., 2002). Unless otherwise

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