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Endothelial microsomal prostaglandin E synthase-1 facilitates neurotoxicity by elevating astrocytic Ca^{2+} levels

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ABSTRACT

Recurrent seizures may cause neuronal damage in the hippocampus. As neurons form intimate interactions with astrocytes via glutamate, this neuron–glia circuit may play a pivotal role in neuronal excitotoxicity following such seizures. On the other hand, astrocytes contact vascular endothelia with their endfeet. Recently, we found kainic acid (KA) administration induced microsomal prostaglandin E synthase-1 (mPGES-1) and prostaglandin E_2 (PGE_2) receptor EP3 in venous endothelia and on astrocytes, respectively. In addition, mice deficient in mPGES-1 exhibited an improvement in KA-induced neuronal loss, suggesting that endothelial PGE_2 might modulate neuronal damage via astrocytes. In this study, we therefore investigated whether the functional associations between endothelia and astrocytes via endothelial mPGES-1 lead to neuronal injury using primary cultures of hippocampal slices. We first confirmed the delayed induction of endothelial mPGES-1 in the wild-type (WT) slices after KA-treatment. Next, we examined the effects of endothelial mPGES-1 on Ca^{2+} levels in astrocytes, subsequent glutamate release and neuronal injury using cultured slices prepared from WT and *mPGES-1* knockout mice. Moreover, we investigated which EP receptor on astrocytes was activated by PGE_2. We found that endothelial mPGES-1 produced PGE_2 that enhanced astrocytic Ca^{2+} levels via EP3 receptors and increased Ca^{2+}-dependent glutamate release, aggravating neuronal injury. This novel endothelium–astrocyte–neuron signaling pathway may be crucial for neuronal damage after repetitive seizures, and hence could be a new target for drug development.

1. Introduction

Neurons and astrocytes form intimate networks in the brain. Astrocytes can modulate synaptic transmission through release of glutamate (Volterra and Steinhauser, 2004; Haydon and Carmignoto, 2006; Perea and Araque, 2007), which contributes to epileptiform discharges (Kang et al., 2005; Tian et al., 2005) and neuronal synchrony (Fellin et al., 2004). In addition, glutamate release from astrocytes plays a crucial role in delayed neuronal injury after status epilepticus (Ding et al., 2007), and subsequent reactive astrogliosis is associated with seizure progression and neuronal death (Cole-Edwards et al., 2006). Thus, astrocytes have an important role in neuronal damage after seizures.

Prostaglandin E_2 (PGE_2) is the most crucial mediator involved in inflammatory processes. PGE_2 biosynthesis is triggered by one of two cyclooxygenase (COX) enzymes, COX-1 or COX-2 (Kulmacz and Wang, 1995; Smith et al., 1996). Following the COX enzymes, three terminal PGE_2-synthesizing enzymes (PGESs) have been shown to convert PGH_2 to PGE_2. First, constitutively expressed cytosolic PGES couples to COX-1 (Tanioka et al., 2000). Second, microsomal prostaglandin E synthase-1 (mPGES-1) (Jakobsson et al., 1999) is inducible by inflammatory stimuli (Ek et al., 2001; Yamagata et al., 2001; Engblom et al., 2003), kainic acid (KA)-induced seizures (Takemiya et al., 2010), pilocarpine-induced

Abbreviations: PGE_2, prostaglandin E_2; COX, cyclooxygenase; PGES, PGE_2-synthesizing enzyme; mPGES-1, microsomal prostaglandin E synthase-1; KA, kainic acid; TMT, trimethyltin; $[Ca^{2+}]_i$, intracellular Ca^{2+}; WT, wild-type; *mPGES-1*$^{-/-}$, *mPGES-1* knockout mice; GFAP, glial fibrillary acidic protein; SR101, sulforhodamine 101; GDH, L-glutamate dehydrogenase; NAD, β-nicotinamide adenine dinucleotide hydrate; v.W., von Willebrand; NeuN, neuronal nuclei; BAPTA-AM, 1,2-bis (2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid, tetraacetoxymethyl ester; DL-TBOA, DL-threo-beta-benzyloxyaspartic acid; PI, propidium iodide; ACSF, artificial cerebrospinal fluid; NMDAR, NMDA receptor.

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seizures (Turrin and Rivest, 2004) or cerebral ischemia (Ikeda-Matsuo et al., 2006) in the brain and is functionally coupled to COX-2 (Yamagata et al., 2001). Last, the other mPGES, mPGES-2, may function following COX-2 synthesis (Bosetti et al., 2004), however it is not essential for PGE_2 biosynthesis following inflammatory stimulation (Jania et al., 2009).

In the brain, neuronal COX-2 expression is increased immediately after seizures (Yamagata et al., 1993; Takemiya et al., 2003) and enhances neuronal excitability (Takemiya et al., 2003, 2007). In addition, a large amount of PGE_2 is primarily produced by COX-2 in the late phase after treatment with kainic acid (KA) or trimethyltin (TMT), and exacerbates neuronal loss elicited by KA or TMT (Kawaguchi et al., 2005; Takemiya et al., 2006; Shirakawa et al., 2007). Furthermore, we recently found that mPGES-1 was co-expressed with COX-2 in venous endothelial cells to produce PGE_2 8–24 h after injection with KA, and aggravated KA-induced neuronal death (Takemiya et al., 2010). However, the mechanism underlying neuronal damage via endothelial PGE_2 remains uncertain.

Exogenous PGE_2 immediately evokes Ca^{2+}-dependent glutamate release from astrocytes (Bezzi et al., 1998); therefore, astrocytes may be activated by endogenous PGE_2 and elevate intracellular Ca^{2+} ($[Ca^{2+}]_i$) levels directly through the PGE_2 receptor EP. Our recent study demonstrated that KA increased the late induction of the EP receptor subtype EP3 on astrocytic endfeet (Takemiya et al., 2010). Moreover, astrocytes can modulate synaptic transmission through the release of glutamate (Volterra and Steinhauser, 2004; Haydon and Carmignoto, 2006; Perea and Araque, 2007), which plays a crucial role in delayed neuronal injury after seizure (Ding et al., 2007). We hypothesized that endothelial PGE_2 produced late by mPGES-1 activates EP3 on astrocytes directly and elevates astrocytic $[Ca^{2+}]_i$ levels, which evokes sustained glutamate release that leads to neuronal damage.

To verify this hypothesis, we performed astrocytic Ca^{2+} imaging using primary cultured hippocampal slices 17 h after treatment with KA. The current study shows that KA-induced mPGES-1 activates Ca^{2+} signaling in astrocytes through EP3 and enhances glutamate release, leading to excitotoxic neuronal injury. To our knowledge, this study is the first to demonstrate that endothelial cells regulate the astrocyte–neuron network via PGE_2 under pathophysiological conditions such as neuronal injury.

2. Materials and methods

2.1. Animals and materials

Wild-type (C57BL/6J; WT) and *mPGES-1* knockout mice (*mPGES-1*$^{-/-}$) were used. Mice were housed four or five per cage in a room maintained at 24 ± 2 °C with a standard 12 h light/dark cycle. The experimental protocols were approved by the Animal Care and Use Committees of the Tokyo Women's Medical University and Tokyo Metropolitan Institute for Neuroscience.

Rabbit anti-mPGES-1, the PGE_2 monoclonal enzyme immunoassay (EIA) kit and PGE_2 were purchased from Cayman Chemical. KA, mouse monoclonal anti-glial fibrillary acidic protein (GFAP)-Cy3 antibody, Dulbecco's modified Eagle's Medium, sulforhodamine 101 (SR101), L-glutamate dehydrogenase (GDH) and β-nicotinamide adenine dinucleotide hydrate (NAD) were from Sigma–Aldrich. KA was used at 0.1 μM for primary culture experiments. Monoclonal mouse anti-human von Willebrand (v.W.) factor was from Dako. Mouse anti-rat CD11b was from AbD Serotec and mouse anti-neuronal nuclei (NeuN) monoclonal antibody was from Millipore. FITC-labeled tomato lectin (*Lycopersicon esculentum*) was purchased from Vector Laboratories. Neurobasal medium, B-27 supplement, 1,2-bis (2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid, tetraacetoxymethyl ester (BAPTA-AM) and Fluo-4 acetoxymethyl ester were from Invitrogen and DL-threo-beta-benzyloxyaspartic acid (DL-TBOA) was from Tocris. ONO-AE-248 (EP3 agonist), ONO-AE3-240 (EP3 antagonist), ONO-DI-004 (EP1 agonist), ONO-8711 (EP1 antagonist), ONO-AEI-259-01 (EP2 agonist) and ONO-AE3-208 (EP4 antagonist) were kindly donated by ONO Pharmaceuticals (Osaka, Japan).

2.2. Primary culture of hippocampal slices and Ca^{2+} imaging

Hippocampal slices were prepared from 6- to 7-day-old postnatal WT and *mPGES-1*$^{-/-}$ mice as previously described (Stoppini et al., 1991; Harris-White et al., 1998). The hippocampi were cut to 400 μm thick slices using a McIlwain Tissue Chopper (TC752) and placed on a membrane (Millicell-CM 0.4 μm, MILLIPORE). Slices were cultured in neurobasal medium supplemented with B-27 and 2 mM L-glutamine at 35 °C in 5% CO_2 and the medium was changed twice a week. Ca^{2+} imaging in astrocytes in slice cultures was performed with a laser confocal microscope. The $[Ca^{2+}]_i$ levels in astrocytes were evaluated with Fluo-4 and SR101 (Fig. 2B). Fluo-4 was prepared using a modification of a previous report (Hirase et al., 2004). In brief, 0.8 μl of the Fluo-4-containing solution was applied to the surfaces of the hippocampal slice cultures with a micropipette. After being loaded for 45 min at 35 °C, astrocytes were identified by the application of 250 nM SR101 for 10 s and washed (Hirase et al., 2004; Nimmerjahn et al., 2004).

2.3. Quantification of the number of Fluo-4-labeled astrocytes in hippocampal slices

To optimize the KA administration conditions to elevate the astrocytic $[Ca^{2+}]_i$ levels, hippocampal slice cultures were exposed to 0.1 μM KA for 2, 4, 6, 8, 12, 17, 20 and 24 h, and the astrocytic $[Ca^{2+}]_i$ levels were visualized with Fluo-4 and SR101 (Supp. Fig. 1). Similarly, hippocampal slices were exposed to 0.1, 1, 10 and 100 μM KA for 17 h, and the astrocytic $[Ca^{2+}]_i$ levels were observed (Supp. Fig. 2). The cells stained with both Fluo-4 and SR101 were counted within a 300 μm × 150 μm rectangle (see Fig. 1C).

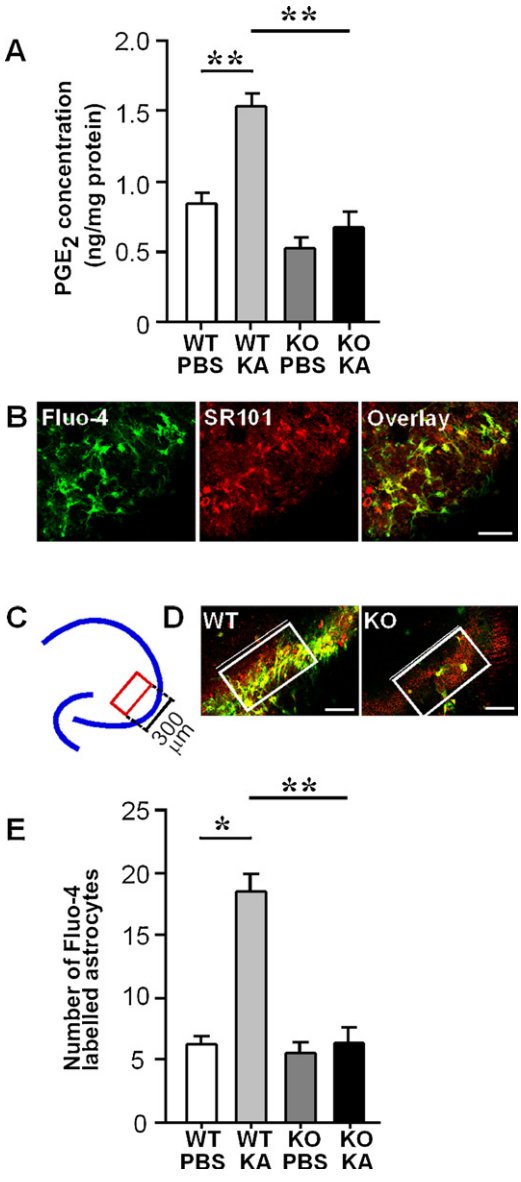

Fig. 1. mPGES-1 produces PGE_2 and increases Ca^{2+} levels in astrocytes, glutamate release and neuronal damage after KA. (A) PGE_2 concentrations in cultured slices from WT and *mPGES-1*$^{-/-}$ slices after the addition of KA or PBS for 17 h (n = 5–6). (B) Astrocytic Ca^{2+} imaging with Fluo-4 and SR101 in WT slices. (C) The rectangle (150 μm × 300 μm) in the CA3 region for counting the cells. (D) Ca^{2+} imaging in WT (left) and *mPGES-1*$^{-/-}$ slices (right). (E) The numbers of Fluo-4 labeled astrocytes within the rectangular areas in WT and *mPGES-1*$^{-/-}$ slices treated with PBS or KA (n = 5). $^{*}p < 0.0001$, $^{**}p < 0.001$.