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Endothelial prostaglandin E_2 regulates neuronal injury after seizure via activation of astrocytes

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ABSTRACT

Astrocytes interact closely with neurons via glutamate; this astrocyte-neuron circuit may play a pivotal role in synaptic transmission. In addition, astrocytes contact vascular endothelial cells (ECs) with their end-feet; therefore, ECs may have some role in regulating neuronal activity via astrocytes in the brain. In our studies, we found that kainic acid (KA) microinjection induced the expression of microsomal prostaglandin E synthase-1 (mPGES-1) in venous ECs and the expression of the prostaglandin E_2 (PGE_2) receptor EP3 on astrocytes. Moreover, endothelial mPGES-1 exacerbated KA-induced neuronal injury in the mouse brain. In *in vitro* experiments, mPGES-1 produced PGE_2, which increased astrocytic Ca^{2+} levels and Ca^{2+}-dependent glutamate release, thus aggravating neuronal injury. We found ECs had a role under pathological conditions and brain ECs are not merely a physiological barrier between the blood and brain; instead, they may also act as a signal transducer or amplifier. Moreover, the endothelium-astrocyte-neuron signaling pathway may be crucial for driving neuronal injury elicited by repetitive seizures and may be a new therapeutic target for epilepsy.

Introduction

Prostaglandin E_2 (PGE_2) is an important modulator in inflammation. In the brain, PGE_2 is associated with neuroinflammation because PGE_2 is involved in pathological processes such as seizure and cerebral ischemia[1,2]. PGE_2 is sequentially synthesized from arachidonic acid by cyclooxygenase (COX) and PGE_2 synthase (PGES). Inducible COX-2 expression is known to be associated with acute neurotoxicity[1-4] and is also involved in delayed proinflammatory activities, which aggravate the neuronal damage found in neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), multiple sclerosis (MS) and Alzheimer's disease (AD)[4,5]. We find that COX-2 is induced in non-neuronal cells late after seizure and facilitates neuronal injury in the hippocampus[6].

In this review, we show the induction of microsomal prostaglandin E synthase-1 (mPGES-1) in brain endothelial cells (ECs) as well as the role of endothelial mPGES-1 in neuronal loss in the hippocampus after seizures. Furthermore, we present a novel mechanism for exacerbation of neuronal injury by PGE_2 derived from endothelial mPGES-1 and discuss the intercellular signaling pathway among endothelia, astrocytes and neurons in the process.

Induction of COX-2 and mPGES-1 in the brain

PGE_2 is synthesized by mPGES-1 coupling with COX-2 in brain ECs in lipopolysaccharide (LPS)-induced fever[7], and mPGES-1 is also co-induced with COX-2 during fever or inflammation[8-11]. Moreover, we find

that mPGES-1 is induced in the hippocampus after epileptic seizures caused by kainic acid (KA) microinjection[12]. KA is an analogue of the excitatory amino acid glutamate, and it is used in research to investigate the mechanisms of hippocampal neuronal loss after seizures because it induces generalized convulsion and causes neuronal loss in the hippocampus after seizures[13]. Unilateral KA microinjection induces COX-2 in bilateral neurons in the hippocampi, but in ipsilateral blood vessels both at 8 h and 24 h after KA injection[6]. Moreover, mPGES-1 is also localized in the blood vessels at 8 h, lasting until 24 h after KA microinjection[12]. Double immunostaining for both mPGES-1 and von Willebrand factor (an endothelial cell marker) shows that mPGES-1 is induced with COX-2 in the ECs for 48 h after the microinjection[12]. Finally, neuronal loss is caused in the KA microinjection side[6], therefore we judge that PGE_2 synthesized by endothelial COX-2 and mPGES-1 facilitates neuronal injury in the hippocampus. Meanwhile, general injection of KA or pilocarpine causes general convulsion and induces COX-2 protein 18 h or 24 h after injection in hippocampal neurons. In addition COX-2 inhibitor blocks the neuronal injury[14,15], suggesting that neuronal COX-2 has an effect to facilitate neuronal injury after strong seizure.

Endothelial mPGES-1 exacerbates neuronal injury

PGE_2 produced in ECs could have a direct effect on the adjacent astrocytes because brain ECs are surrounded by astrocytic end-feet[16]. In addition, several lines of evidence indicate that prostaglandin E receptors (EPs) are present on astrocytes, and exogenous PGE_2 immediately evokes Ca^{2+}-dependent glutamate release from astrocytes[17]; therefore, astrocytes may be directly activated by endogenous PGE_2 to elevate the intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) through the EP receptors. Furthermore, astrocytes can modulate synaptic transmission through the release of glutamate[18-20], which may stimulate delayed neuronal injury after seizures[21]. Therefore, we hypothesized that PGE_2 produced by endothelial mPGES-1 directly activated EP receptors on astrocytes, elevating the astrocytic $[Ca^{2+}]_i$, subsequently evoking sustained glutamate release and ultimately facilitating neuronal injury.

We found that the PGE_2 concentration was significantly elevated by KA treatment in cultured hippocampal slices from wild type (wt) mice, but that increase was not observed in slices from *mPGES-1* knockout mice (*mPGES-1*$^{-/-}$)[22]. The astrocytic $[Ca^{2+}]_i$ was significantly higher in the hippocampal CA3 region in the wt slice cultures than in the *mPGES-1*$^{-/-}$ slice cultures[22]. These results suggest that the PGE_2 derived from mPGES-1 upregulates the astrocytic $[Ca^{2+}]_i$ in the hippocampal CA3 region.

Next, we examined the effects of each EP receptor antagonist and agonist on the $[Ca^{2+}]_i$ in astrocytes in the KA-treated wt and *mPGES-1*$^{-/-}$ slice cultures. An EP3 receptor antagonist[23] decreased the $[Ca^{2+}]_i$ in astrocytes in the KA-treated wt slices[22], conversely, an EP3 receptor agonist[23] increased the $[Ca^{2+}]_i$ in astrocytes in the KA-treated *mPGES-1*$^{-/-}$ slices, suggesting that the EP3 receptor has a crucial role in astrocytic Ca^{2+} elevation[22]. EP3 immunoreactivity was rarely detected in the astrocytes in naive control mice; in contrast, it was enhanced in the astrocytic end-feet with swelling after KA microinjection[22]. In the *mPGES-1*$^{-/-}$ mice, the end-feet also showed swelling, but the EP3 immunoreactivity was not increased as much as in the wt mice[22], indicating that the EP3 receptor was locally induced by KA in hippocampal astrocytes, which might receive PGE_2 from ECs. Previous publications have reported that *EP3* mRNA is expressed in cultured astrocytes[24] and that EP3 protein expression is induced in astrocytomas by interleukin-1β[25]. These findings indicate that astrocytic EP3 receptors may be upregulated under pathological conditions, and endothelial PGE_2 may directly activate EP3 receptors on astrocytic end-feet in neurotoxic brain diseases, such as epileptic seizures. PGE_2 also acts on other three receptors, namely EP1, EP2 and EP4, and activation of their receptors has been found to contribute to PGE_2-mediated neurotoxicity[26]. Block of EP1 receptor reduces proinflammatory responses and neuronal damage in the hippocampus after KA injection in mice[27]. Moreover, selective EP2 antagonism by small molecules prevents up-regulation of COX-2 in microglia, leading to reduce neuronal injury induced by pilocarpine[28]. In addition, EP4 receptor-associated protein promotes proinflammatory activation of microglia which modulates neuronal damage[29]. Activation of these receptors is concerned with regulation of neuronal injury by PGE_2.

Furthermore, we observed that treatment with KA for 17 h dramatically increased the level of glutamate release in the wt slices but not in the *mPGES-1*$^{-/-}$ slices[22]. To verify whether mPGES-1 regulates hippocampal neuronal death, we stained the cells with propidium iodide (PI). The results showed greater PI uptake in the CA3 region of the wt slices than in that of the *mPGES-1*$^{-/-}$ slices[22]. This significant increase in PI uptake in the wt slices suggests that neuronal injury may be enhanced by mPGES-1, which regulates the Ca^{2+}-dependent glutamate release from astrocytes.

We next added exogenous PGE_2 to the *mPGES-1*$^{-/-}$ slices to validate the above findings on the endogenous PGE_2. PGE_2 enhanced the astrocytic $[Ca^{2+}]_i$ in the CA3 region[22]. Moreover, PGE_2 caused an increase in the glutamate concentration and exacerbated the PI uptake in the CA3 region[22]. These results indicate that the PGE_2 derived from mPGES-1 is an important mediator that regulates

neuronal injury. Exogenous PGE_2 also increased neuronal $[Ca^{2+}]_i$ in co-cultures with astrocytes, but the increase was not found without astrocytes (unpublished data). In addition, the $[Ca^{2+}]_i$ increase in neurons was observed to follow the $[Ca^{2+}]_i$ increase in astrocytes (unpublished data). These results suggest that PGE_2 indirectly increases the neuronal $[Ca^{2+}]_i$ via the astrocytic $[Ca^{2+}]_i$ increase and subsequent glutamate release. Finally, we investigated whether this PGE_2-evoked glutamate release occurred in a Ca^{2+}-dependent manner. A membrane-permeable Ca^{2+} chelator, BAPTA-AM, diminished the increase in the $[Ca^{2+}]_i$ in the astrocytes in the wt slices and abolished the increase in glutamate concentration[22]. Moreover, BAPTA-AM partially ameliorated the neuronal injury in the CA3 region, suggesting that CA3 neuronal injury is locally regulated by Ca^{2+}-dependent glutamate release from neighboring astrocytes[22]. Together, these results suggest that the PGE_2 produced by endothelial mPGES-1 activates the astrocytic EP3 receptors to elevate the $[Ca^{2+}]_i$ in astrocytes, causing Ca^{2+}-dependent glutamate release and facilitating neuronal injury[22].

Intercellular signaling pathway among endothelia, astrocytes and neurons

Neuron-to-astrocyte signaling controls arterial blood flow in the brain[30-32]. Conversely, there is also mounting evidence for dynamic astrocyte-to-neuron interactions; for example, astrocytes modulate synaptic transmission[18-20]. The interactions are also involved in neuronal synchrony[33] and epileptic discharges[14,34], which contribute to a delayed neuronal injury after seizures[21]. Neurons are vulnerable to glutamate in the hippocampus, and it is thought to be mediated by *N*-methyl-D-aspartate (NMDA) receptors (NMDARs)[35]. In particular, glutamate release from astrocytes activates the extrasynaptic NMDAR subunit NR2B, which induces neuronal currents[21] or triggers neuronal loss[21,36,37]. This suggests that extrasynaptic NR2B receptors play crucial roles in the neurotoxicity caused by the glutamate released from astrocytes. Conversely, neuronal glutamate activates astrocytic mGluR5 to cause an increase in $[Ca^{2+}]_i$ in astrocytes, which may in turn release glutamate and generate feedback to extrasynaptic NR2B[21]. Thus, the neuron-astrocyte circuit may amplify the glutamate signaling, which aggravates neuronal excitotoxicity following seizures.

In this review, we propose an advanced mechanism for excitotoxicity via ECs and astrocytes. We demonstrated that endothelial mPGES-1 regulated Ca^{2+} signaling in astrocytes and Ca^{2+}-dependent glutamate release, consistent with the findings that application of exogenous PGE_2 propagated astrocytic $[Ca^{2+}]_i$ and evoked Ca^{2+}-dependent glutamate release[17]. However, PGE_2 alone did not increase astrocytic $[Ca^{2+}]_i$ (unpublished data); therefore, PGE_2 may require another factor, such as a concomitant activation of astrocytic EP3, to elevate $[Ca^{2+}]_i$ in astrocytes. Brain ECs are not merely a physiological barrier between the blood and brain; instead, they may also act as a signal transducer or amplifier. In particular, we found ECs had a role under pathological conditions, such as in epileptic seizure. The interaction among neurons, astrocytes and ECs may be key to understanding the processes of seizure-induced neuronal injury in epilepsy.

Conclusions

PGE_2 is synthesized by inducible mPGES-1 and COX-2 in vascular ECs in response to KA microinjection. In addition, endothelial PGE_2 activates astrocytic EP3 receptor to elevate $[Ca^{2+}]_i$ levels in astrocytes, causing Ca^{2+}-dependent glutamate release which stimulates neuronal injury. This is a new mechanism underlying neuronal injury regulated by ECs; therefore, this review emphasizes that brain ECs act as a signal transducer or amplifier, especially, under pathological conditions, such as epileptic seizure. The analysis of the interactions among neurons, astrocytes and ECs provides a better understanding of the processes of seizure induced neuronal injury and will facilitate the development of new treatments.

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Conflict of Interest

The authors declare no conflict of interest.

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