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increased neuronal $[Ca^{2+}]_i$ in co-cultures with astrocytes, but the increase was not found without astrocytes (unpublished data). In addition, the $[Ca^{2+}]_i$ increase in neurons was observed to follow the $[Ca^{2+}]_i$ increase in astrocytes (unpublished data).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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