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Review

Reactive astrocytes and therapeutic potential in focal ischemic stroke

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ABSTRACT

Astrocytes are specialized and the most abundant cell type in the central nervous system (CNS). They play important roles in the physiology of the brain. Astrocytes are also critically involved in many CNS disorders including focal ischemic stroke, the leading cause of brain injury and death in patients. One of the prominent pathological features of a focal ischemic stroke is reactive astrogliosis and glial scar formation. Reactive astrogliosis is accompanied with changes in morphology, proliferation, and gene expression in the reactive astrocytes. This study provides an overview of the most recent advances in astrocytic Ca^{2+} signaling, spatial, and temporal dynamics of the morphology and proliferation of reactive astrocytes as well as signaling pathways involved in the reactive astrogliosis after ischemic stroke based on results from experimental studies performed in various animal models. This review also discusses the therapeutic potential of reactive astrocytes in focal ischemic stroke. As reactive astrocytes exhibit high plasticity, we suggest that modulation of local reactive astrocytes is a promising strategy for cell-based stroke therapy.

Introduction

Astrocytes are the most numerous glial cell type in the central nervous system (CNS). In a normal brain, there are two major types of astrocytes: fibrous and protoplasmic astrocytes. They can be found in white matter such as the corpus callosum and in grey matter such as the cortex. Glial fibrillary acidic protein (GFAP) is primarily expressed in the thick main processes and has been considered as a 'pan-astrocyte' marker (Brenner, 2014), but its expression levels are higher in the fibrous astrocytes than in the protoplasmic astrocytes. Transcriptome analysis found that the Aldh1L1gene is most widely and homogenously expressed in the astrocytes, while immunostaining with an anti-Aldh1L1 antibody revealed that the Aldh1L1 protein is highly expressed in the astrocyte cell body and its extensive processes (Cahoy et al., 2008). Thus, Aldh1L1 is now considered as a new 'pan-astrocyte' marker.

It has been long recognized that astrocytes play a critical role in the physiology and are very important for overall brain architecture as well as function. Astrocytes can maintain ionic homeostasis by acting as a potassium (K^+) sink (Djukic et al., 2007). Astrocytes can remove synaptically released glutamate by their glutamate transporters to avoid glutamate excitotoxicity (Huang et al., 2004; Bergles et al., 1999). Astrocytes also mediate Ca^{2+} signaling and intercellular waves through the stimulation of different G-protein coupled receptors (GPCRs) via phospholipase-C/inositol 1,4,5-triphosphate (PLC/IP_3) pathway *in vivo*. These GPCRs include metabotropic glutamate receptors (mGluRs) (Ding et al., 2007; Fellin et al., 2004; Sun et al., 2013), P2Y receptors (Ding et al., 2009; Thrane et al., 2012; Sun et al., 2013; Wang et al., 2006; Nizar et al., 2013), $GABA_B$ receptors ($GABA_B$Rs) (Ding et al., 2009; Meier et al., 2008), noradrenergic receptors (Bekar et al., 2008; Ding et al., 2013; Paukert et al., 2014). Due to the intimate physical contact with synapses, astrocytes are considered as a part of the 'tripartite' synapse where they can listen and talk to the synapse by regulating Ca^{2+} increase in response to neuronally released transmitters and by gliotransmitter release (Haydon, 2001). We have also known that astrocytes and the blood vessels have intimate anatomic relationship. This was further confirmed by studies using fluorescence imaging and electron microscopy showing that astrocyte endfeet almost completely cover the cerebral vascular surface (Simard et al., 2003; Petzold et al., 2008; Mathiisen et al., 2010; Kacem et al., 1998). Astrocytic Ca^{2+} signaling is involved in functional hyperemia from *in vitro* studies of brain slice preparations (Zonta et al., 2003; Mulligan and MacVicar, 2004; Gordon et al., 2008); however, its role in the regulation of cerebral blood flow (CBF) *in vivo* is controversial as suggested by results from studies using type 2 IP_3 receptor knockout mice (Jego et al., 2014; Takata et al., 2013; Nizar et al., 2013; Bonder and McCarthy, 2014).

Growing evidence indicates that astrocytes are heterogeneous in morphology, molecular expression, and physiological function under normal conditions (Zhang and Barres, 2010; Matyash and Kettenmann, 2010). Morphologically, protoplasmic astrocytes, and fibrous astrocytes are different. Protoplasmic astrocytes are complex (sponge like) and

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highly branched with numerous fine processes and their endfeet wrap around blood vessels, while fibrous astrocytes are less complex and have thicker and less branched processes (Wilhelmsson et al., 2006; Bushong et al., 2002). Numerous studies have found that different genes are expressed among different subsets of astrocytes *in vivo* (Zhang and Barres, 2010). GFAP expression is higher in the astrocytes in corpus callosum, but it is expressed in astrocytes in the cortex at lower levels (Xie et al., 2010). Electrophysiologically, astrocytes exhibit a different current–voltage relationship with one type of astrocytes, known as outward rectifying astrocytes, when compared to the other known as variably rectifying astrocytes (Zhou and Kimelberg, 2000). Astrocytes also exhibit different properties of Ca^{2+} signaling *in vivo*. Two-photon (2-P) *in vivo* Ca^{2+} imaging has shown that astrocytes in the cortical layer 1 (L1) nearly doubled the Ca^{2+} activity compared to the astrocytes in L2/3 in anaesthetized rats; moreover, Ca^{2+} signals in the processes in the same astrocyte were asynchronous in L1 while those in L2/3 were more synchronous (Takata and Hirase, 2008). The morphological, molecular, and functional heterogeneity of astrocytes indicates a diversity among astrocytes and the complex physiological and pathological roles that astrocytes play in the CNS.

Astrocytes respond to different neurological diseases through a common phenomenon of GFAP upregulation, a process termed as reactive astrogliosis. Severe CNS injuries such as stroke, traumatic brain injury (TBI), and spinal cord injury (SCI), as well as neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis (ALS) all cause a massive upregulation of GFAP. Therefore, GFAP is considered a reliable marker to characterize reactive astrocytes. However, given the different causes and onsets of diseases, the temporal and spatial changes of the reactive astrocytes are different. For example, in the AD brain, due to slow disease progression, the reactive astrocytes are more evenly distributed and do not form glial scars. While after ischemic stroke or SCI, reactive astrocytes in the peri-infarct region express higher GFAP and eventually form glial scar, which establishes both a physical and biochemical barrier that separates dead and vital tissues. Thus, the properties of reactive astrocytes in chronic neurodegenerative diseases are different from those seen in acute conditions like focal ischemia and SCI. Although similar phenomena, such as glial scar formation, is observed in both focal ischemia and SCI, in experimental animal models of SCI, the injury occurs in the large area of white matter rather than in grey matter as seen in ischemic strokes. The functions and role of reactive astrocytes have been much more extensively studied in SCI than in the focal ischemic stroke (for reviews, see Burda and Sofroniew, 2014; Sofroniew, 2009; Sofroniew and Vinters, 2010; Silver and Miller, 2004; Rolls et al., 2009). Thus, this article will review the dynamic changes in astrocytic Ca^{2+} signaling, morphology, and proliferation of reactive astrocytes. The article also examines the distribution of reactive astrocytes surrounding the ischemic core, i.e., in the penumbra, in experimental animal models of focal ischemic stroke. Discussion then focuses on the signaling pathways involved in reactive astrogliosis after focal ischemia followed by the therapeutic potential of reactive astrocytes in ischemic stroke. For extensive reviews of reactive astrocytes in various aspects in different neurological diseases, readers are advised to consult a few detailed reviews (Burda and Sofroniew, 2014; Sofroniew and Vinters, 2010; Escartin and Bonvento, 2008; Anderson et al., 2014).

Dynamics of reactive astrocytes in the penumbra after focal ischemia

Focal ischemic stroke, resulting from the blockage of cerebral blood vessels, leads to cell death and brain damage and causes human disability and death (Stapf and Mohr, 2002). After the onset of ischemia, astrocytes undergo numerous pathological alterations over time, including rapid swelling (Nedergaard and Dirnagl, 2005; Barber and Demchuk, 2003; Swanson et al., 2004; Li et al., 2014) and enhanced Ca^{2+} signaling (Ding et al., 2009). Astrocytes can also become reactive following ischemia. The hallmark of reactive astrogliosis is the morphological changes and the increased expression levels of GFAP (Nedergaard and Dirnagl, 2005; Panickar and Norenberg, 2005). Reactive astrocytes eventual form glial scar in the penumbra that demarcates the ischemic core (infarction) from healthy tissue (Hayakawa et al., 2010; Barreto et al., 2011b; Shimada et al., 2011; Bao et al., 2012; Li et al., 2014). In addition to reactive astrogliosis, focal ischemic stroke is also accompanied by other temporally and spatially dependent changes. As the clinical aim of stroke therapy is to salvage the penumbral cells, understanding the spatial and temporal changes of astrocytes at molecular and cellular levels will provide therapeutic strategy insights.

Ca^{2+} signaling in astrocytes after ischemia

A few *in vitro* and *in vivo* studies have demonstrated how ischemia induces enhanced Ca^{2+} signaling in different stages after ischemia onset (for an extensive review, see Ding, 2013, 2014a). Using acute brain slice preparations and an oxygen-glucose deprivation (OGD) model, Duffy and MacVicar (1996) found that a short episode (5 min) of simultaneous hypoxia and hypoglycemia can induce an intracellular Ca^{2+} increase within an average of 7.5 min, and it takes 2.5 min to reach a peak. After reoxygenation, astrocytic Ca^{2+} will remain elevated for a highly variable period of time ranging from several minutes to 1 h. In the absence of extracellular Ca^{2+}, astrocytic Ca^{2+} increase can still be observed with a relatively consistent duration. Electrophysiological recordings have shown that hypoxia and hypoglycemia also depolarize astrocytes (Duffy and MacVicar, 1996). The data from brain slice experiments suggest that astrocytes can mediate Ca^{2+} increase from the internal store release and influx of voltage-dependent Ca^{2+} influx. Alternatively, internal Na^{+} accumulation can lead to Ca^{2+} influx through reverse operation of the sodium-calcium exchanger (NCX). In another study using brain slices, OGD induced slow inward currents (SICs), which were mediated by extrasynaptic *N*-methyl D-aspartate (NMDA) receptors in rat hippocampal CA1 pyramidal neurons (Dong et al., 2013). Moreover, dialysis of 1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), an Ca^{2+} chelator, into the astrocytes reduced SIC frequency after OGD, implying that astrocytic Ca^{2+} activity stimulate extrasynaptic NMDA receptors. Using Ca^{2+} imaging, it was found that astrocytes are quiescent in spontaneous Ca^{2+} activity in brain slices under normal conditions, but astrocytes exhibits frequent Ca^{2+} elevations during OGD. Surprisingly, within the 10 min of OGD, more than 60% of astrocytes have exhibited Ca^{2+} elevations. Moreover, a majority of astrocytes displayed more than two Ca^{2+} transients. To further demonstrate the importance of astrocytic Ca^{2+} activity in ischemia, slice preparations from type 2 IP_3R knockout mice were used. Astrocytes exhibited rare Ca^{2+} transients during OGD and low-frequency SICs in CA1 neurons in IP_3R2 knockout mice as compared with wild-type (WT) mice.

Using a photothrombosis (PT)-induced ischemia model and *in vivo* two-photon (2-P) imaging, Ding et al. (2009) found that astrocytes exhibit enhanced Ca^{2+} signaling characterized as intercellular Ca^{2+} waves starting ~20 min after PT. Both amplitude and frequency of astrocytic Ca^{2+} signal were significantly increased compared to relative quiescent Ca^{2+} signaling prior to ischemia. In addition, the magnitude of a Ca^{2+} signal was smaller in the penumbral region than the magnitude of a Ca^{2+} signal in the ischemic core. An important feature of the Ca^{2+} signals was that most of them started and returned to the basal level at the same time among the astrocytes in the imaging field, i.e., they exhibited a high degree of synchrony. Pharmacological study has suggested that glutamate and GABA released into the extracellular space following PT stimulated intercellular waves among astrocytes. The results represent the first direct evidence of an astrocytic Ca^{2+} response after ischemia in an *in vivo* setting. Acute increase in astrocytic Ca^{2+} signals have also been observed after a single vessel occlusion by PT (Zheng et al., 2013). Acute response of astrocytes with enhanced

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