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Invited review

Glial calcium signaling in physiology and pathophysiology[1]

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Abstract

Neuronal-glial circuits underlie integrative processes in the nervous system. Function of glial syncytium is, to a very large extent, regulated by the intracellular calcium signaling system. Glial calcium signals are triggered by activation of multiple receptors, expressed in glial membrane, which regulate both Ca^{2+} entry and Ca^{2+} release from the endoplasmic reticulum. The endoplasmic reticulum also endows glial cells with intracellular excitable media, which is able to produce and maintain long-ranging signaling in a form of propagating Ca^{2+} waves. In pathological conditions, calcium signals regulate glial response to injury, which might have both protective and detrimental effects on the nervous tissue.

Introduction

Recent advances in experimental investigations of glial networks challenged the neuronal doctrine that, since 1894[1], dominated our perception of brain function. The new concept, which regards brain as a dynamically interconnected reticular network of internally connected glial syncytium and synaptically connected neuronal circuits, is now emerging[2,3]. This new concept also includes new and more complex pathways of intercellular signaling, which can be executed in two modes, the wiring and the volume transmission[4–7]. The wiring transmission represents a fast local and unidirectional mode of intercellular signaling executed through chemical and electrical synapses. The volume transmission is a slow and global route for cellular communications, which takes either extracellular (diffusion of signaling molecules in the extracellular space) or intracellular (diffusion of messengers and metabolites in the cellular syncytium connected by gap junctions) route. These two modes of transmission operating in concert permit extreme sophistication in information processing within neural circuits.

Glial cells, which account for 60% of all cells in the nervous system of rodents and approximately 90% of all neural cells in humans, perform a wide variety of vital functions[8]: radial glial cells and "stem" astrocytes control neurogenesis, neural cell development and migration; astroglia divide gray matter into neuronal-glial-vascular units and are actively involved in synaptic transmission; oligodendrocytes provide for axonal myelination; and microglia act as a main defense system in the nervous tissue. On a molecular level, integration within neuronal-glial networks very much relies on a specific signaling system that uses Ca^{2+} ions as the universal cellular messengers (Figure 1)[9–11]. Indeed, the Ca^{2+} signaling system controls integration in both synaptically connected neuronal synaptic networks (by controlling neurotransmitter release from presynaptic terminals and secretion of neurohormones) and within glial syncytium (by providing the glia with the means of long-range signaling by propagating calcium waves).

Glial cells are also heavily involved in many types of brain diseases[12]. Insults to the nervous system trigger specific glial reactions represented by reactive astrogliosis, Wallerian degeneration and activation of microglia. These glial responses are of critical importance for the neural pathology. Conceptually, it is important to remember that astroglial cells can outlive neurones. Moreover, astrocytes

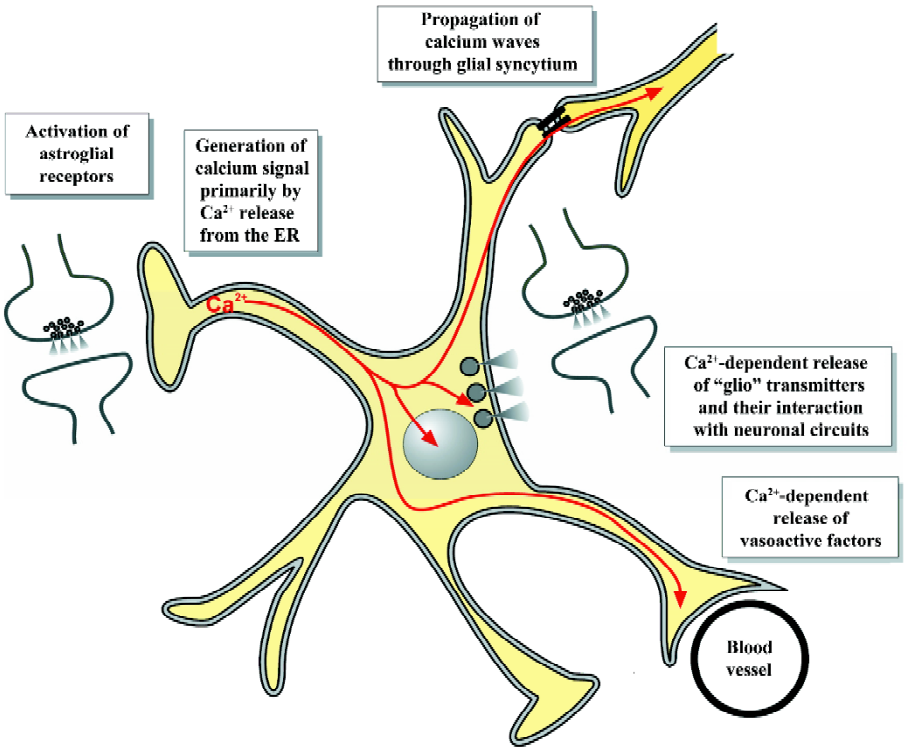

Figure 1. Calcium signaling and integration in glial-neuronal-vascular units.

very often are activated in the presence of dying or already dead neurones, whereas neurones cannot survive without astroglial support. Likewise, failure of oligodendrocytes leads to the development of demyelinating diseases, which severely affect axonal function. Finally, failure of microglial defense leaves the central nervous system (CNS) open to infection-induced damage.

In this essay I shall overview the main properties of physiological glial calcium signaling and give several examples of glial calcium involvement in neuropathology.

Glial calcium signaling

Ca^{2+} signaling as a substrate for glial excitability Although glial cells are non-excitable from the classical physiological point of view (they are unable to generate regenerative action potentials), they are able to respond to external stimulation with generation of intracellular Ca^{2+} signals. The latter result from activation of Ca^{2+} fluxes either through plasmalemma or through intracellular membrane, which form a specific organelle known as endoplasmic reticulum (ER). This intracellular membrane, the endomembrane, acts as an excitable media[13–16]. The excitability of the endomembrane is determined by a specific complement of intracellular Ca^{2+} channels and endo(sarco)plasmic reticulum calcium ATPases (SERCA), which act as Ca^{2+} pumps that transport Ca^{2+} against the steep concentration gradient from the cytosol into the ER lumen[17]. The intra-ER free Ca^{2+} concentration (or intraluminal Ca^{2+} concentration, $[Ca^{2+}]_L$) reaches several hundreds of micromoles and creates an electro-driving force aimed at the cytosol[18–20]. Intracellular Ca^{2+} channels residing in the endomembrane are represented by several families: (1) ryanodine receptors (RyRs) directly controlled by cytosolic Ca^{2+}; (2) inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$), sensitive to both cytosolic Ca^{2+} and second messenger $InsP_3$; and (3) possibly NAADP receptors[21–23]. Importantly, free Ca^{2+} ions within the lumen of ER act as the main regulators of both Ca^{2+} release channels and SERCA Ca^{2+} pumps. Decrease in $[Ca^{2+}]_L$ activates SERCA uptake and inhibits Ca^{2+} release channels, whereas elevation of $[Ca^{2+}]_L$ slows down the SERCA pumping and increases the sensitivity of Ca^{2+} release channels to stimulation[24,25]. Local Ca^{2+} release from the ER, manifested in a form of "sparks" or "puffs"[14], creates microdomains of an increased Ca^{2+} concentration, which in turn are able to recruit neighbouring Ca^{2+} release channels and thus produce a propagating wave of excitation of the endomembrane. This "propagating" Ca^{2+} wave serves as a substrate for long-range signaling in glial syncytium; as glial Ca^{2+} waves are able to cross the cell-to-cell boundaries and travel a long distance though astroglial

networks[26–29]. Mechanisms responsible for the generation of propagating Ca^{2+} waves are complex and involve diffusion of $InsP_3$ through gap junctions, connecting astroglial cells, and release and extracellular diffusion of transmitters such as ATP or glutamate (Ref 30–33 and Figure 2).

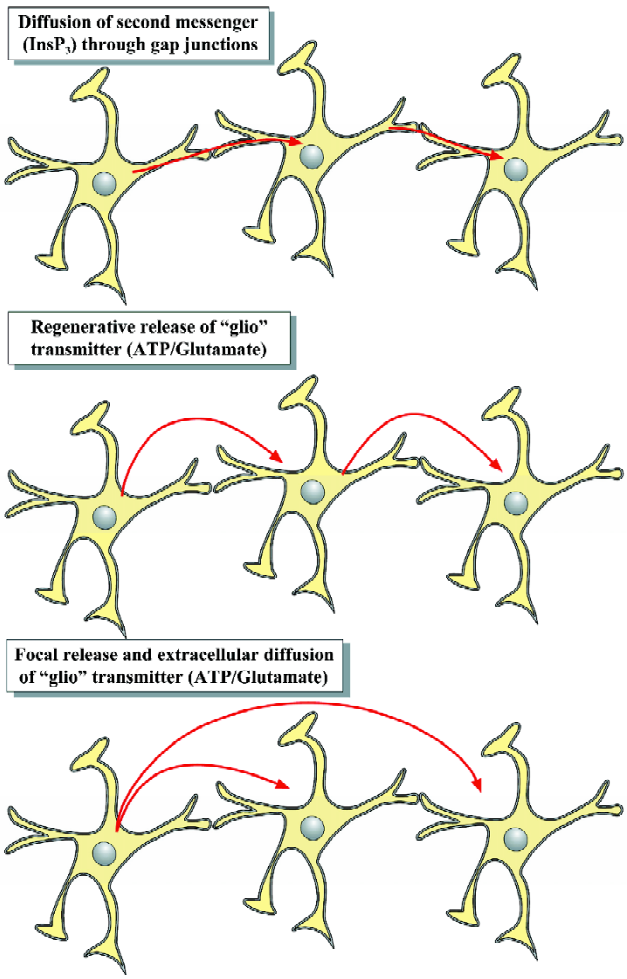

Figure 2. Mechanisms of Ca^{2+} wave propagation through glial syncytium.

Glial Ca^{2+} and integration in neuronal-glial networks The incoming signaling, activating glial cells are represented by neurotransmitters, released during neuronal synaptic communications. Astrocytes form structures that closely enwrap the majority of synapses in grey matter. As a consequence, the astroglial compartment can be regarded as an inseparable part of the synapse (the concept of tripartite synapse[34–36]). Astrocytes express all types of known neurotransmitters receptors, and most importantly expression of these receptors shows remarkable regional heterogeneity, being tuned to specifically sense neurotransmitters, released in the adjoining synapses[3,29,36–41]. Many glial receptors belong to the metabotropic variety, and their activation leads to formation of $InsP_3$ and subsequent Ca^{2+} release from the ER. In addition, astrocytes also express ionotropic receptors, which can produce cell depolarization and also serve as a route for plasmalemmal Ca^{2+} entry. Astrocytes of the brain grey matter usually express glutamate and P2 purinoreceptors[41–44], which is not surprising as glutamate and ATP are involved in excitatory neurotransmission in the majority of central synapses. Synaptic activity triggers activation of glial receptors and generation of both ionotropic and cytosolic Ca^{2+} responses in virtually all types of astrocytes studied in brain *in situ* preparations[45–47]. Astroglial calcium signals in turn control regulated exocytosis of "glio" transmitters, which include glutamate, *D*-serine, ATP and perhaps other neuro-active substances[48–53]. These "glio" transmitters directly affect neurones residing within astroglial territories and might either directly excite them, or modulate the ongoing synaptic transmission[54–58]. Calcium signals traveling within astrocytes also create a functional link between neuronal activity and local circulation, by triggering a release of vasoactive substances from astrocyte endfeet, which engulf brain capillaries[59,60].

To conclude, glial calcium signaling acts as a molecular mechanism for integration within glial syncytium and between glial and neuronal circuits. Quite naturally, this powerful signaling system also plays an important role in neuropathology.

Glial calcium in neuropathology

Brain ischaemia Disruption or insufficiency of the blood flow in the CNS causes considerable damage to the nervous tissue. The blood flow in the brain can be compromised either by blood vessel rupture, which leads to haemorrhage, or by a restraint of blood supply to the whole brain or to its parts, which is generally known as brain ischemia. The latter can be global (when brain blood supply stops because of, for example, heart failure) or focal (when regional blood flow is reduced or ceased completely due to vascular occlusion). Focal brain ischemia is manifested by stroke. Both global and focal ischemia trigger neural cell death, which primarily results from the limitation of oxygen supply (hypoxia or anoxia) as well as with restrictions in delivery of metabolic substrates.

Focal ischemia produces spatially distinct damage areas. At the very centre of the ischemic zone lies the infarction

core, surrounded by ischemic penumbra. The core is formed almost instantly after the cessation of blood flow and is represented by an area of pan-necrosis of all neural elements; formation of penumbra is much slower and might take several days.

All types of glial cells are affected by the ischemic insult and their reaction, to a very considerable extent, determines the outcome of the stroke. Acute ischemia rapidly kills both neurones and oligodendrocytes, but astroglial cells are generally more resistant. The swift death of neurones and oligodendrocytes is largely mediated through glutamate excitotoxicity: the cells located within the infarction core rapidly lose the ability to maintain transmembrane ion gradients and undergo anoxic depolarization. This results in Ca^{2+} influx, which in turn triggers a massive release of glutamate. The latter activates ionotropic receptors and further exacerbates cellular Ca^{2+} overload. Persistent and severe elevation in $[Ca^{2+}]_i$ in turn compromises mitochondria, induces oxidative stress and activates numerous proteolytic enzymes. All of these processes result in necrosis. In neurones, the primary route for Ca^{2+} entry after exposure to glutamate is mediated by NMDA receptors[61–63]. In oligodendrocytes and oligodendrocyte precursors, Ca^{2+} enters through both Ca^{2+}-permeable AMPA/kainate receptors[64] and through NMDA receptors, recently discovered in oligodendroglia[65–67]. Oligodendroglial death during ischemic insults is rapid and can cause severe demyelination syndromes, such as periventricular leucomalacia[68] or Binswanger's disease[69].

Astrocytes are considerably less sensitive to glutamate excitotoxicity. Moreover, astroglial cells which, by virtue of high expression of glutamate transporters, are the main sink of glutamate in the brain (for example, up to 80% of glutamate released during synaptic activity is accumulated by astrocytes) form the chief defensive system against glutamate toxicity. In cell culture conditions, astrocytes can survive for up to 12 h in conditions of oxygen and glucose deprivation[70]. In contrast, *in vivo* the gray matter astrocytes are more vulnerable to ischemia, and relatively prolonged occlusion of blood flow (approximately 2 h) causes prominent astroglial death[71]. The astroglial responses to acute ischemia are mainly manifested by a rapid increase in $[Ca^{2+}]_i$, which starts to develop several minutes after induction of ischemia[72,73]. This cytoplasmic Ca^{2+} rise results from both plasmalemmal Ca^{2+} entry and Ca^{2+} release from the ER calcium stores. Interestingly, the magnitudes of ischemia-induced $[Ca^{2+}]_i$ elevation was much larger in *in situ* (brain slice) preparations compared to isolated cells[73].

Notwithstanding these rapid reactions, astroglial cells can survive for a long time in the penumbra, where they may, to a considerable degree, determine the progression of the infarction and its outcome. First, astroglial cells can maintain anaerobic glycolysis and thus supply adjacent neurones with an energy supply in a form of lactate[74]. Second, astrocytes act as powerful scavengers of reactive oxygen species, as they contain high concentrations of glutathione and ascorbate, which represent principal anti-oxidants in the CNS. The ability of astrocytes to protect neurones against reactive oxygen species has been clearly demonstrated *in vitro*: neuronal–astroglial cultures were much more resistant to injury produced by superoxide or hydrogen peroxide, compared to purified neuronal cultures[75]. Third, astroglial networks are instrumental for extracellular potassium buffering[76], and by dispersing potassium from the affected areas astrocytes protect neural tissue against severe depolarization. Finally, astroglial calcium signals might be instrumental in initiating reactive gliosis, which can determine the neurological outcome in a post-ischemic period.

The role played by astrocytes in brain ischemia can, however, be detrimental, and in certain conditions astroglial reaction can exacerbate the nerve tissue damage. In particular, astrocytes might play a leading role in propagation of the cell damage through penumbra and even in triggering death of neurones in areas distal to the ischemic core. In particular, propagating Ca^{2+} waves, initiated by focal ischemia, can spread through astroglial syncytium and cause release of glutamate and some other, still unidentified, pathological factors; these factors, in their turn, can cause neural cell death, thus leading to expansion of the infarct[77–79]. In principle, astroglial networks seem to be the main players in propagation of damaging signals from infarction core to the surrounding tissue, as indeed signaling through neuronal circuits can be excluded because neuronal excitability is lost after even a mild reduction of cerebral blood flow. Of course, the extent and velocity of infarct expansion will depend on many factors, affecting astroglial function (such as the degree of tissue acidification, and depth of metabolic failure), yet the astroglial performance might very likely determine the progression of brain ischemia.

Spreading depression The spreading depression (initially described in 1944 by Aristide Leào[80]) is a wave of severe neuronal depolarization that spreads through the gray matter at a velocity of approximately 1.5–7.5 mm/min. This propagating wave of depolarization can occur in normal brain tissue as a consequence of sharp local increase in extracellular K^+ concentration or release of glutamate, both of which can be triggered by excessive neuronal activity. This wave of spreading depression, for example, can lead to migraine attacks[81]. The spreading depression can also be initiated by

mechanical or ischemic damage, and as such it is often observed in the penumbra surrounding the infarction core. In the latter case, the waves of spreading depression play an important role in the expansion of the infarct through the penumbra, so that every next wave increases the damage[82,83]. In the normal, non-ischemic tissue spreading depression does not trigger cell damage, although it might initiate activation of microglia[84] and mild reactive astrogliosis[85].

The precise mechanisms of spreading depression are not fully understood; most likely its initiation results from several factors acting in concert. Nonetheless, it can be linked to astroglial calcium waves with similar propagation velocity. Interestingly, disruption of astroglial gap junctions inhibits both propagating Ca^{2+} waves and the propagation of waves of spreading depression[82].

Epilepsy Epilepsy is a severe and often debilitating neurological disorder, manifested by seizures, accompanied with motor abnormalities and disturbances of consciousness and behavior. The cellular substrate of epilepsy is represented by a spontaneous and synchronous depolarization of all neurones within the epileptic foci, known as a paroxysmal depolarization shift (PDS). The PDS is generated by simultaneous activation of postsynaptic glutamate receptors and lasts for 50–200 ms. The actual nature of the synchronous release of glutamate remained elusive for many years, although in 1986 it was suggested that it might have a non-synaptic origin[86,87]. Experimental evidence gained very recently[88–90] indicates that astroglial cells might act as a source of glutamate, which induces PDS and epileptiform neuronal activity.

The experimental PDS, induced by several interventions (superfusion of brain slices with low Ca^{2+} solutions, addition of K^+ channel blocker 4-aminopyridine, or inhibition of $GABA_A$ receptors by bicuculline) was developing in conditions of synaptic isolation, that is, when neuronal firing was completely blocked by tetrodotoxin[90]. This experiment suggested that the glutamate, which triggers PDS, can be released from non-neuronal structures. Indeed, when Ca^{2+} waves were induced in astrocytes (by selective liberation of UV-sensitive "caged" Ca^{2+}), this resulted in release of glutamate and initiation of PDS. Two-photon confocal video-imaging of the cortical structures also demonstrated that, usually, astroglial Ca^{2+} waves preceded PDS and neuronal discharges and moreover, intraperitoneal injection of anti-epileptic drugs reduced both astroglial Ca^{2+} waves and neuronal PDS[90]. Obviously, the experimental epileptic models cannot completely reproduce the disease situation, although it is known that epilepsy is accompanied with massive reactive gliosis that develops even before any neurodegenerative changes and appearance of fully developed seizures[91,92]. It could be that reactive astrocytes have altered Ca^{2+} signaling, which might further add to the pathogenesis of epileptic seizures. Introduction of astrocytes into the epileptic circuit can explain the precise synchronization between many neurones. Every astrocyte in gray matter could be connected with up to 100 000 synapses within its domain, therefore, glutamate released from the astroglial cell might reach all the neurones virtually simultaneously.

Alzheimer's disease Alzheimer's disease (AD), initially described by Alois Alzheimer as a malignant dementia in a 51-year-old woman[93], is manifested by: (1) occurrence of β-amyloid protein deposits in the form of plaques; (2) intraneuronal accumulation of abnormal tau-protein filaments in the form of neuronal tangles; and (3) profound neuronal loss leading to severe dementia. Histopathology of AD is also characterized by prominent reactive astrogliosis and activation of microglia[94].

The effects of astroglia in the progression of AD can be both protective and detrimental. Astroglial cells act as the natural scavenger of amyloid proteins. Reactive astrocytes can migrate towards deposits of β-amyloid, then accumulate and degrade them[95]. In contrast, astrocytes can mediate neuronal injury: overloading of astroglial cells with β-amyloid triggers their degeneration. As a consequence, the astrocytes withdraw their processes from the neuronal membranes and synapses residing within their territory, exacerbating neuronal damage[94]. Astroglial calcium waves can provide a specific mechanism for neurotoxicity in AD. Recently, it was shown that superfusion of neuronal-glial co-cultures with β-amyloid triggers $[Ca^{2+}]_i$ oscillations in astrocytes, without inducing neuronal Ca^{2+} signals. These Ca^{2+} waves, maintained for long periods (as long as β-amyloid was present in the culture media) induced degenerative changes and eventual death of neighboring neurones. Inhibition of astroglial Ca^{2+} signals by culture treatment with thapsigargin was neuroprotective[96,97].

Conclusions

Glial calcium signaling acts as a powerful system that provides for integration within glial syncytium and between glial and neuronal circuits. These calcium signals arise in response to the stimulation of glial receptors by neurotransmitters and neurohormones; they give birth to propagating Ca^{2+} waves that spread though glial networks and control release of "glio" transmitters, which signal to neuronal networks. In pathological conditions, calcium signaling is intimately involved in the regulation of glial responses, which

have both protective and detrimental effects on the nervous tissue.

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