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Models of astrocytic Ca^{2+} dynamics and epilepsy

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Abstract

Neurons have been the focus of neuroscience research. Only recently, however, astrocytes, a subset of glial cells, have been on the neurobiology "radar" owing to their Ca^{2+} excitability, which allows them to signal to other astrocytes and neurons. This review summarizes the models for studying astrocytic Ca^{2+} dynamics and the consequential Ca^{2+}- dependent glutamate release, which plays a role in astrocytic-neuronal signaling and have been implicated in epilepsy.

Introduction

Basic research in astroglial biology for the past two decades has changed how we view the mammalian brain. Astrocytes are not just housekeeping cells which remove glutamate from the synapses [1], supply trophic factors [2] and offer metabolic support to neurons [3], but they are also players in cell-cell signaling in the central nervous system (CNS) [4]. Astrocytes present themselves as an integral component of synaptic transmission, where these cells not only can sense neuronal glutamatergic transmission, but, by their ability to release this transmitter, they can also modulate and control the strength of synaptic transmission. Hence, astrocytes exhibit Ca^{2+} excitability in the form of Ca^{2+} oscillations and intra- and inter-cellular waves in response to neuronal signals [5], since they express receptors for neurotransmitters and neuroligands [6]. Furthermore, astrocytic changes in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) can result in Ca^{2+}-dependent release of gliotransmitters such as glutamate [7]. In recent years, the Ca^{2+} excitability of astrocytes, and their ability to release/uptake glutamate has implicated astrocytic role, not only in physiology, but also in pathophysiological states of the brain, including epilepsy [8]. This disease alone affects an estimated number of 50 million people worldwide [9]; it does not only cause significant pain, suffering and disability, but, as patients become disabled and eventually eligible for Medicaid, there is a corresponding increase in the expenditure of public dollars, both state and federal.

We discuss *in vitro*, *in vivo*, and *in silico* models that are presently available for studying the role of astrocytic Ca^{2+} excitability in (patho)physiology of mammalian CNS. *In vitro* models encompass dissociated astrocytic cultures, cultured organotypic slices from various brain structures, and acute brain slices. *In vivo* modes are represented by the use of anesthetized rodents, while *in silico* models deal with computational biology.

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In vitro models of astrocytic Ca^{2+} excitability, consequential Ca^{2+}-dependent glutamate release, and glutamate-mediated bidirectional signaling between astrocytes and neurons

Roger Y. Tsien and colleagues made a lasting contribution to the astrocytic research, and neurobiology in general, when they generated fluorescent Ca^{2+} indicators for optical measurements of cellular free Ca^{2+} [10]. Soon after, it became apparent that astrocytes exhibit intra- and intercellular Ca^{2+} dynamics that are sensitive to neuronal signals. Cornell-Bell *et al*. made the seminal finding that dissociated cultures of astrocytes can respond to exogenously added neurotransmitter glutamate by changing their $[Ca^{2+}]_i$, displaying oscillations and step responses [11]. Additionally, elevated Ca^{2+} propagated in waveforms among many astrocytes suggesting a long-distance communication between these cells. Similar responses have been subsequently observed in cultured organotypic [12] and acute slices from the rat hippocampus [13] and visual cortex [14]; astrocytes responded to the neuronal communication where glutamate was released into and escaped (spilled-over) from the synaptic cleft.

In addition to Ca^{2+} excitability due to activation of glutamatergic receptors (GluRs), *in vitro* studies also revealed that astrocytes expressed plasma membrane receptors for many other ligands, including adenosine triphosphate (ATP), epinephrine, norepinephrine, γ-aminobutyric acid, acetylcholine and bradykinin to mention some [6]. Binding of these ligands to their astrocytic receptors result in $[Ca^{2+}]_i$ increase primarily due to the release of Ca^{2+} from internal stores, although entry of this ion from extracellular space plays a role. Majority of above listed ligand-gated receptors are coupled to G-proteins that activate phospholipase C to produce inositol 1,4,5-trisphosphate (IP_3). Binding of IP_3 to its receptor on the endoplasmic reticulum (ER) membrane, results in the opening of an associated channel to release Ca^{2+} into astrocytic cytosol, which subsequently can cause glutamate release.

There has been some discrepancy in identifying the expression of receptors, and proteins in general, when using various *in vitro* astrocytic models. For instance, culturing may result in expression of the exocytoctic protein synaptosome-associated protein of 25 kDa (SNAP-25) which would not be produced by astrocytes *in vivo* [15]. Additionally, the protein function in astrocytes may be altered by culturing of these cells. For example, the purinergic $P2X_7$ receptor, an ATP-gated receptor channel, has been identified in dissociated culture, and, albeit with variable success, also in acute slices and *in vivo*. However, while its functionality has been confirmed in dissociated cultures of astrocytes, it seems to be absent in hippocampal astrocytes of rat and human acute slices [16]. Thus, supplementary practices should be implemented to verify the presence and activity of proteins *in vitro* models of astrocytes. A model of freshly (acutely) isolated astrocytes, which provides information of cellular properties without the changes in gene expression [17,18], can partially alleviate this problem.

In vitro studies revealed an exciting consequence of astrocytic Ca^{2+} dynamics; astrocytes can exocytotically, in a Ca^{2+}-dependent manner, release gliotransmitters [19], such as glutamate, which can then signal to adjacent neurons. Hence, purified and cultured astrocytes of visual cortex released glutamate in response to bradykinin [20]. If neurons were co-cultured with astrocytes, glutamate released by astrocytes caused responses in adjacent neurons, observed as an increase in neuronal $[Ca^{2+}]_i$. Follow-up studies in cultured hippocampal neurons and astrocytes identified this glutamate-mediated astrocyte-neuron signaling pathway to underlie modulation of spontaneous and action-potential evoked synaptic transmission [21,22], leading to the concept of “tripartite synapse”[23]; astrocytes are an integral element of a synapse together with neuronal pre- and post-synaptic sites. The findings from these initial studies in dissociated cultures have been confirmed in acute slices, as reviewed elsewhere [4,24]. We briefly outline three recent studies highlighting astrocytic ability to control synaptic strength.

In acute slices, astrocytes modulate the perforant path-granule cell synapses in the hippocampal dentate molecular layer [25]. Here, stimulation of astrocytes in acute slices increased the frequency but not the amplitude of spontaneous excitatory post-synaptic currents (EPSCs) and the amplitude of action potential-evoked EPSCs on granule cells, implying that there is a presynaptic mechanism of action. Pharmacology suggested that the NR2B subunit-containing *N*-methyl-D-aspartic acid (NMDA) receptors were responsible for this enhancement of synaptic transmission. Similarly, in a CA1 region of hippocampus a G-protein coupled protease-activated receptor 1 (PAR-1) stimulation of astrocytes to induce glutamate release was shown to enhance NMDA mediated EPSCs, miniature EPSCs and excitatory post-synaptic potentials in neurons of hippocampal slices [26]. Furthermore, glutamate released from astrocytes, caused by the liberation of Ca^{2+} from a Ca^{2+} cage delivered to astrocytes, surrounding CA3-CA1 hippocampal synapses in acute slices, lead to the increased probability of glutamate release from pre-synaptic neurons by activating their pre-synaptic metabotropic GluRs (mGluRs) [27]. This short-term plasticity became persistent when it was temporally coincident with post-synaptic depolarization. Thus, release of glutamate from astrocytes can lead to induction of long-term potentiation at CA3-CA1 hippocampal synapses *via* activation of neuronal pre-synaptic mGluRs.

To have a full partnership in this glutamate-mediated bidirectional communication between astrocytes and neurons, astrocytes should be able to initiate this signaling based on their intrinsic activity. Indeed, using freshly prepared slices from ventrobasal thalamus and Ca^{2+} imaging, Parri *et al.* [28] showed that astrocytes display intrinsic $[Ca^{2+}]_i$ oscillations, which were not driven by neuronal activity. These spontaneous $[Ca^{2+}]_i$ oscillations in astrocytes could initiate Ca^{2+} waves in adjacent astrocytes, and also cause NMDA receptor -mediated neuronal excitability.

Undeniably, *in vitro* models contributed greatly to our understanding of astrocytic Ca^{2+} excitability, astrocytic Ca^{2+} -dependent glutamate release and bidirectional signaling between astrocytes and neurons. These models offered manipulation of single cells or a small group of cells. Additionally, the use of *in vitro* models lead to realization that commercially available acetoxymethyl (AM) esters of fluorescent indicators, that are cell permeant, preferentially load to astrocytes over neurons in slices, unlike in cultured cells. Such loadings are now routinely used to monitor astrocytic Ca^{2+} dynamics *in vivo* using rodents. Similarly, acute slices have been used in testing the advent of novel genetic approaches that, for example, selectively manipulate exocytotic release from astrocytes [29]. This provides a good starting point to rigorously test the contribution of glutamate release pathways in astrocytes, which in near future could be performed *in vivo* to critically assess the role of these cells in health and disease.

In vivo models of astrocytic Ca^{2+} dynamics

Imaging of astrocytic Ca^{2+} dynamics *in vivo* was spurred: (i) by the ability to preferentially deliver AM forms of fluorescent indicators into astrocytes, and (i) by the application of two-photon fluorescence microscopy which allows observation of fluorescent indicators in superficial cortical layers. For *in vivo* imaging, during anesthesia of rats or mice, a craniotomy is performed to make a cranial window, typically 2-4 mm in diameter. After dura removal, an AM form of Ca^{2+} indicator is topically applied to the pial surface which leads to loading of astrocytes with the indicator. Agarose is then applied to fill the cranial window that is covered by the glass coverslip to minimize brain pulsation. At this juncture imaging using two-photon confocal microscopy is performed.

Astrocytic Ca^{2+} dynamics in live animals appear to have similar properties as seen in cultured cells. Hirase *et al.* showed that in anesthesized rats, astrocytes exhibited $[Ca^{2+}]_i$ fluctuations [30] similar to those previously observed in astrocytes from cell cultures and slice tissues.

Additionally, they showed that astrocytic Ca^{2+} excitability may be correlated to neuronal activity. Here, local application of bicuculline to induce neuronal hyperactivity caused an increase in the number of astrocytes that exhibited transient Ca^{2+} increases, referred to as "spikes." These findings support the notion that there is neuron-astrocyte communication in intact brain.

Wang *et al*. used sensory stimulation to study astrocytic Ca^{2+} dynamics *in vivo* [31]. They deflected the whiskers of anesthesized adult mice and measured astrocytic $[Ca^{2+}]_i$ in layer 2 of the barrel cortex while simultaneously recorded neuronal activity using the local field potentials. In unstimulated animals, they observed that astrocytes rarely exhibited spontaneous Ca^{2+} oscillations at their cell bodies, but displayed frequent oscillations at the processes. However, after mechanical stimulation of the whiskers, the astrocytes responded with delayed global increases in $[Ca^{2+}]_i$. It appears that the number of astrocytes that responded to whisker stimulation depended on the strength of external stimulus. Using pharmacological agents, they found that the astrocytic responses were mediated by glutamate released from neurons, which the acted upon astrocytic mGluRs. These finding are of fundamental importance for brain function since they indicate that astrocytes, like neurons, are under the influence of environmental signals (whisker deflection).

Astrocytic Ca^{2+} dynamics *in vivo* induced by sensory stimulation are not limited to the whisker-barrel cortex system, but are also displayed in somatosensory cortex of mice upon painful peripheral stimulation (foot shock) of the contralateral limb. This signaling required the activity of locus coeruleus neurons, which by releasing norepinephrine activated astrocytic α-adrenergic receptors and lead to increases of astrocytic $[Ca^{2+}]_i$ [32].

In silico models of astrocytic-neuronal signaling

The *in silico* models of astrocytic Ca^{2+} dynamics and astrocytic-neuronal signaling are in early stages of development. The limitation to their development, in part, comes from the lack of experimental data necessary for refined computational models. Nonetheless, the first steps implementing *in slico* models to this rapidly growing area of neuroscience have been made. Volman et at. developed a simple biophysical model in which Ca^{2+} dynamics of astrocytes in a tripartite synapse modulate the synaptic transmission [33]. Here, the authors used self-synaptic contacts, so called autapses, where a neuron makes a synapse to itself; such synapses have been used for *in vitro* assessment of glutamate-mediated astrocyte-neuron signaling [34].

Nadkarni and Jung generated a computational model in which astrocytic glutamate release regulates internal Ca^{2+} stores located on the presynaptic neuron *via* activation of mGluRs [35]. This model predicts that such astrocytic coupling to a synapse represents a form of synaptic plasticity where a low fidelity synapse can become a high fidelity synapse. It should be noted that this prediction has been subsequently demonstrated as an induction of long-term potentiation at CA3-CA1 hippocampal synapses *via* activation of neuronal pre-synaptic mGluRs [27] due to temporally coincident post-synaptic depolarization and Ca^{2+}-dependent glutamate release from a perisynaptic astrocyte.

Besides *in silico* models for the roles of astrocytic Ca^{2+} dynamics in physiology, there have been attempts to utilize the computational modeling for astrocytic roles in pathophysiology, especially epilepsy. Silchenko and Tass devised a computational model in which astrocytic Ca^{2+} dynamics and consequent glutamate release caused paroxysmal depolarization shifts (PDS) in neighboring neurons which resulted in a synchronous action potential discharge (spiking) activity [36]. This particular model is interesting because it predicts proposed mechanisms of how astrocytes may participate in epilepsy [37].

Models of astrocytic Ca^{2+} dynamics in epilepsy

Recent lines of evidence suggest astrocytic involvement in epilepsy: (i) functional alterations of specific astrocytic membrane channels, receptors and transporters have been discovered in this disorder [8], and (ii) astrocytes by releasing glutamate can synchronize neuronal firing and modulate neuronal excitability and synaptic transmission [4]. Here, we focus on the later component discussing the contribution of astrocytic Ca^{2+} dynamics and consequential glutamate release in epilepsy.

Kang *et al.* showed evidence that Ca^{2+} -dependent glutamate release from astrocytes is involved in the generation of neuronal epileptiform activity [37]. Infusion of exogenous IP_3 into astrocytes of acute hippocampal slices caused an increase in astrocytic $[Ca^{2+}]_i$. Consequent release of glutamate from astrocytes elicited slowly decaying transient inward currents (STC) in neighboring CA1 pyramidal neurons. This neuronal current corresponded to transient depolarizations with the overriding discharges of several action potentials followed by after hyperpolarization potentials. Such electrical events resemble the PDS which characterize interical epileptiform events.

The follow up study from this group further supports the notion that astrocytes are a key element in epilepsy. Tian *et al.* used the A-type K^+ channel blocker 4-aminopyridine to induce epileptiform activity in neurons of acute hippocampal slices [38]. They noted that PDS persisted when they suppressed the synaptic transmission with the voltage-gated Na^+ channel blocker tetrodotoxin (TTX). PDS could be induced by photolysis of caged Ca^{2+} in astrocytes leading to glutamate release which targeted nearby neurons [38]. These findings identify astrocytes as a key player in seizure activity and thus as a target for drug intervention in epilepsy.

In contrast to studies described above, Fellin *et al.* showed that neuronal action potential discharges, rather than Ca^{2+}-dependent glutamate release from astrocytes, are necessary for the generation of epileptiform activity in acute hippocampal slices [39]. They found that astrocytes can be activated during eplileptiform activity, induced by the removal of extracellular Mg^{2+}/application of picrotoxin that blocks inhibitory synapses, and this astrocytic activation can lead to the increased frequency of NMDA-receptor dependent slow-inward currents (SIC; equivalent to STC in ref. [37]), a finding consistent with the above studies. However, even though these SICs could trigger synchronous depolarization and action potential discharges in small groups of neurons, these events did not play a key role in intericatal (PDS)- like eplieptiform activity, but rather could play a role in the duration/strength of ictal (seizure)-like events. The partial discrepancy between these studies could perhaps be attributed to differences in experimental approaches and various agents being used for the induction of epileptiform activity. Nonetheless, astrocytic Ca^{2+} dynamics appear to play a role in pathophysiology of epilepsy.

The unremitting seizure status epilepticus can lead to delayed neuronal death. Utilizing an approach that combined the use of acute slices and *in vivo* imaging, Ding *et al.* demonstrated that this neuronal death is due to increased astrocytic Ca^{2+} excitability [40]. Using pilocarpine to induce status epilepticus they showed that astrocytes have increased Ca^{2+} excitability lasting for 3 days. This astrocytic hyperexcitability lead to release of glutamate that acted upon extrasynaptic NR2B subunit-containing NMDA receptors, whose activation was the probable cause of neuronal death. This study sheds new light into astrocytic role in epilepsy implicating astrocytes as a key component in the induction of neuronal death.

Model comparison

All the models have their distinct advantages and disadvantages as outlined in Table 1. Consequently, they should be utilized accordingly to address scientific questions at hand. As technology advances, however, the opportunities for *in vivo* models will expand. Of particular interest is the recent advent of transgenic animals with the inducible gene expression/deletion under specific astrocytic promoter(s) [29]. This approach has enormous potential in testing the roles of astrocytic Ca^{2+} dynamics and consequential glutamate release on animal behavior.

Model translation to humans

The rodents are presently used as a translation model to humans. Technologically, the experiments on human subjects for monitoring astrocytic Ca^{2+} dynamics *in vivo* are now feasible. While craniotomy in humans is a standard surgical procedure, the imaging using two-photon microscopy can also be performed through the thinned skull without opening the cranial window. The impediment to such experiments, however, presents itself in the application of AM forms of fluorescent indicators, which need first to be approved by the Food and Drug Administration for human use. Meanwhile, it appears prudent to attempt such experiments in non-human primates.

Conclusion

Understanding intracellular Ca^{2+} dynamics in astrocytes requires studying the CNS at different levels of analysis, from the reductionist approach using the cell culture to systemic approach using freely moving animals, thus, combining *in vitro*, *in vivo*, and *in silico* models. It is interesting to note that as interest grows concerning the role of astrocytes in epilepsy, it is the basic research regarding the role of astrocytes in synaptic transmission that have led us to this association between astrocytes and epilepsy. Indeed, to fully understand the pathophysiology of a disease, one must have an understanding of the physiology of the system that the disease attacks. Thus, from observations of Ca^{2+} excitability and Ca^{2+} -dependent glutamate release in astrocytes we have generated alternative approaches to study epilepsy.

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Table 1

Comparison of astrocytic Ca^{2+} dynamics models

	***In vitro* models**	***In vivo* models**	***In silico* models**
Pros	• Study (sub)cellular components of astrocytic Ca^{2+} dynamics • Study astrocytic modulation of synapses	• Assess effect on behavior • Study disease model in whole organism	• Generates predictions that can be tested
Cons	• Abnormal expression of proteins in culture	• Live imaging limited to superficial layers of cortex	• Needs to be revised with new empirical evidence
Best use of model	• Study Ca^{2+} dynamics of single cells	• Study disease model in whole organism	• Provides framework for future experiments
How to get access to the model	• Literature, academic supplier	• Literature, academic supplier	• Literature, academic supplier
Relevant patent	n/a	n/a	n/a
Refs	[11-18,20-22,25-29,34,37-40]	[15,17,18,30-32,40]	[33,35,36]