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Astrocytes and Epilepsy

Jerome Clasadonte and Philip G. Haydon*.[1]

Post-mortem studies of patients with temporal lobe epilepsy show that astrocytes become reactive with altered protein expression. When taken together with the observation that astrocytes release chemical transmitters the idea has developed that these glia contribute to the generation of seizures. In the epileptic brain there is a reduction in expression of an astrocyte-specific enzyme glutamine synthetase (GS) which converts synaptically released glutamate into glutamine, a precursor for glutamate and GABA synthesis. It is shown that selective reactive astrocytosis, and the accompanying loss of GS, leads to reduced synaptic inhibition and increased spread of excitation. In addition, reactive astrocytes exhibit increased expression of adenosine kinase, the enzyme responsible for converting adenosine, an endogenous anticonvulsant, to AMP. Consequently, a reduction in adenosine accompanies reactive astrocytosis. Astrocytes also regulate surface expression of neuronal NMDA receptors and contribute to their excitation through the release of glutamate and D-serine. Astrocytic Ca^{2+} signals, which are dampened by some anticonvulsants, stimulate the release of glial glutamate which can provide excitation to neurons. When this excitatory pathway is taken together with the reduction in GABA- and adenosine-dependent inhibition seen during reactive astrocytosis it is not hard to envision how these support cells might contribute to seizure generation.

INTRODUCTION

Glia, Greek for 'glue', was discovered by Rudolph Virchow, a German anatomist in the mid-nineteenth century. The name reflects the original view that glia played merely a structural or metabolic support role for neurons. Glial cells, especially astrocytes, are much more than 'glue' or merely quiescent, and display their own set of activities. Studies over the last 20 years show that astrocytes perform a series of complex functions that go well beyond the uptake and recycling of neurotransmitters and the buffering of extracellular potassium.[1,2]

Morphologically, astrocytes are characterized by a highly ramified structure of thin processes with which they contact neurons, blood vessels and other astrocytes. Astrocyte-astrocyte contacts mediate gap-junction coupling between adjacent glial cells to form a cellular network called *astrocytic syncytium*.[3] Although astrocytes form a highly interconnected network, they are structurally organized in non-overlapping spatial domains with limited

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interdigitation of processes between adjacent cells.[4,5] Within its own domain of occupancy, each astrocyte can contact tens of thousands of synapses and hundreds of dendrites[4,5](Figure 1A) . The contact between the astrocyte and the neuron is a highly dynamic structure[6,7] and the extent of astrocytic coverage of the neuronal terminals is activity-dependent.[8]

While astrocytes have been considered as non-excitable cells, because unlike neurons they do not fire action potentials,[9] they display a form of excitability that is based on variations of the intracellular Ca^{2+} concentration.[10] Astrocytes express a plethora of receptors for neurotransmitters whose activation leads to increases in intracellular Ca^{2+} concentration that can propagate to neighboring astrocytes as an intercellular Ca^{2+} wave.[10–12] These Ca^{2+} increases promote the release of neuroactive substances, the so-called *gliotransmitters* (Figure 1B). These gliotransmitters can control diverse brain processes, such as vasculature tone[13] and neuronal activity[1,2] and can also modulate mammalian behavior such as sleep.[1,2]

In keeping with the currently accepted concept that astrocytes form an integral and active part of excitatory and inhibitory synaptic transmission and communicate back to synapses,[14] emerging evidence has suggested a critical role for these glial cells in the pathogenesis of neurological disorders such as epilepsy.[15–17] Astrocytes become reactive in the epileptic brain and show changes in the expression of metabolic enzymes such as glutamine synthetase and adenosine kinase leading to modification of neuronal excitability. Astrocytes also release glutamate through a Ca^{2+}-dependent mechanism that can synchronize neuronal firing and modulate neuronal excitability and synaptic transmission. In this chapter, we will focus on current lines of evidence suggesting the involvement of reactive astrocytes and gliotransmission in experimental studies of epilepsy, and possible underlying mechanisms.

Ca^{2+} SIGNALS IN ASTROCYTES

In Vitro and *In Situ* Studies

The development of video imaging techniques and fluorescent indicators of Ca^{2+} has allowed us to observe dynamic spatiotemporal changes in Ca^{2+} concentration[18] in neurons and glial cells simultaneously. Unlike neurons, astrocytes do not produce action potentials, and thus they were thought to be quiescent. However, in the early 1990s, initial cell culture studies reported that the excitatory neurotransmitter glutamate elicited Ca^{2+} elevations in individual astrocytes that can propagate to neighboring astrocytes as an intercellular Ca^{2+} wave involving dozen of cells, suggesting long-distance communication between these cells.[10–12] Further evidence for neuronal activity-dependent Ca^{2+} elevations in astrocytes comes from studies showing that the stimulation of Schaffer collateral in hippocampal slices preparation also increases the intracellular Ca^{2+} concentration in these glials cells.[19]

The synaptic control of astrocytic Ca^{2+} signals is due to the fact that astrocytes express a wide range of functional receptors for different neurotransmitters.[20] Many of these receptors are of the metabotropic type, such as metabotropic glutamate receptor 5 (mGluR5) and metabotropic receptor activated by purines such as P_2Y_1. These receptors are associated with G proteins that, upon activation, stimulate phospholipase C and formation of diacylglycerol and inositol (1,4,5)-triphosphate (IP_3). In turn, IP_3 increases the intracellular concentration of Ca^{2+} through the release of Ca^{2+} from intracellular IP_3-sensitive Ca^{2+} stores.[21] Subsequently, several lines of evidence coming from brain slice preparations demonstrated that excitatory neuronal activity can trigger Ca^{2+} elevations in astrocytes.[21]

In Vivo Studies

Recent studies using two-photon microscopy[22,23] and specific fluorescent dyes that selectively label astrocytes[24] convincingly demonstrate that neuronal activity can trigger Ca^{2+} signals in astrocytes *in vivo*. Using these two revolutionary techniques, Hirase et al.[25] were the first to analyze changes in Ca^{2+} signals in cortical astrocytes

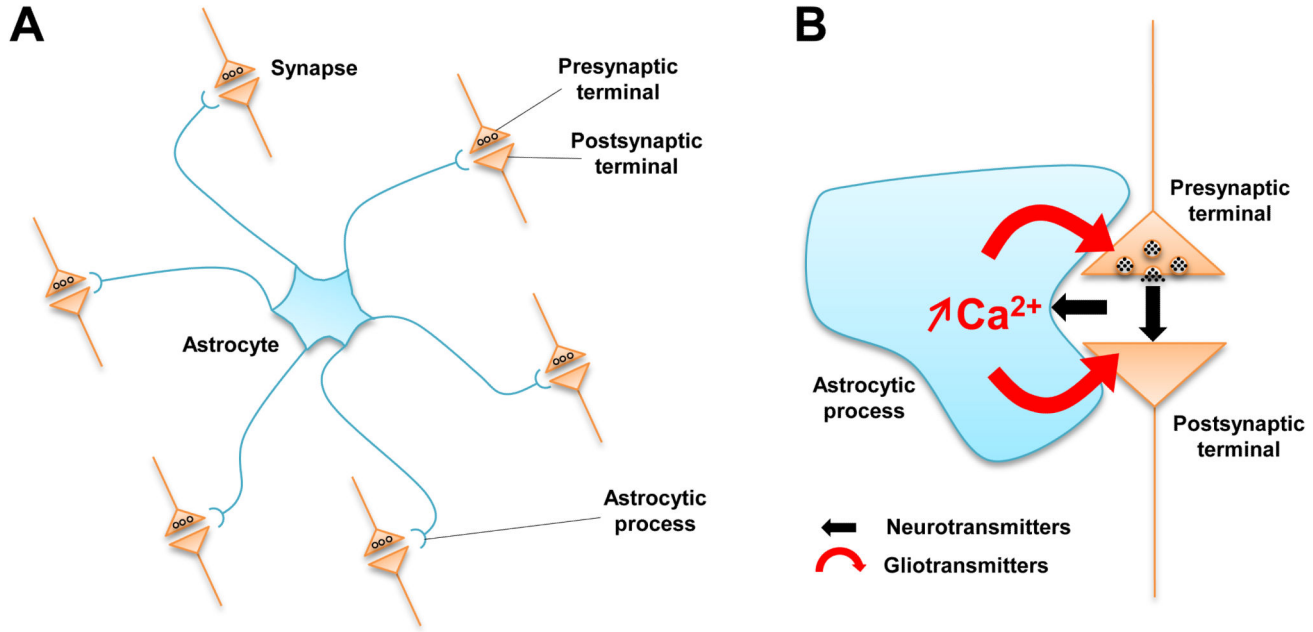

Figure 1. The tripartite synapse. (A) A single astrocyte sends out processes that enwrap a number of synapses. One synapse results from the association between the pre- and postsynaptic terminals. (B) At the synapse, neurotransmitters released from the presynaptic terminal activate an astrocyte which responds with Ca^{2+} elevations. In turn, Ca^{2+} elevations lead to the release of neuroactive substances called *gliotransmitters* which act back to the synapse to regulate the presynaptic function and modulate the postsynaptic response.

from living, anesthetized rat. More than 60% of the imaged astrocytes showed a complex pattern of changes in intracellular Ca^{2+} concentration. However, these changes occured with a relatively low frequency under basal conditions and showed a limited degree of correlation with nearby astrocytes.

The first evidence that astrocytes respond to neuronal activity by increasing their intracellular Ca^{2+} concentration *in vivo* was the observation that the application of gamma-aminobutyric acid A ($GABA_A$) receptor antagonists such as bicuculline[25] or picrotoxin,[26], which increase neuronal activity by triggering epileptic-like discharges, resulted in an increase in Ca^{2+} signaling in cortical astrocytes. Additionally, these Ca^{2+} signals were correlated between pairs of nearby astrocytes,[25] suggesting that *in vivo* neuronal activity leads to synchronous Ca^{2+} signals in multiple astrocytes. Subsequently, sensory stimulation was shown to increase Ca^{2+} signals in astrocytes in anesthetized animals. In the mouse, whisker,[27] limb,[28] and odor stimulation[29] causes Ca^{2+} elevations in astrocytes in the whisker barrel, the primary somatosensory cortex and the olfactory bulb respectively. Generally, these Ca^{2+} increases in astrocytes were delayed by a few seconds compared with the neuronal responses[27,30] and were significantly correlated with the strength of the sensory stimulation.[27] Moreover, a study in ferrets[30] demonstrates that visual stimulation can also induce Ca^{2+} signals in astrocytes in the visual cortex. Interestingly, this study found that astrocytes were even more sharply tuned for stimulus orientation and frequency than neurons.

More recently, neuronal activity in awake, behaving mice was shown to correlate with Ca^{2+} elevations in astrocytes. Two-photon microscopy through a cranial window of an awake, head-restrained mouse allowed to run on a Styrofoam ball was used to visualize Ca^{2+} signals in cortical neurons and astrocytes.[31] Repetitive Ca^{2+} signals were associated with the running behavior and were temporally correlated in multiple astrocytes over a distance of almost 100 μm.[31] Similar studies were performed in the cerebellum, where radial Bergmann glia signals were found to correlate with locomotor behavior and were sensitive to blockade of neuronal activity.[32] Interestingly, in these studies, Nimmerjahn et al.[32] described three different forms of Ca^{2+} signals; one of them

was initiated during locomotor behavior and correlated with changes in blood perfusion, suggesting that these glia networks modulate macroscopic changes in brain dynamics and blood flow.[32]

In vivo pharmacological studies have been performed to elucidate the mechanisms underlying activity-mediated Ca^{2+} signals in astrocytes. *In vivo* application of the mGluR1 or the mGluR5 antagonists LY367385 or 6-methyl-2-(phenylethynyl)-pyridine (MPEP), respectively, reduced the whisker activity-induced Ca^{2+} elevations in astrocytes, while the application of the ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione(CNQX) had no effect on the astrocytic Ca^{2+} response,[27] indicating a role for both mGluR1and mGluR5 but not for ionotropic receptors in astrocyte activation. Additionally, another *in vivo* study has shown that application of the astrocytic glutamate transporter inhibitor DL-threo-β-benzyloxyaspartate (TBOA) reduced Ca^{2+} elevations in astrocytes induced by visual stimulation,[30] suggesting a role for glutamate transporters in the modulation of Ca^{2+} signals in astrocytes.

Together, these *in vivo* results demonstrate that, in response to sensory stimulations, astrocytes exhibit complex and extremely finely tuned intracellular Ca^{2+} signals generated by the synaptic release of glutamate that activates mGluRs and glutamate transporters at the astrocytic surface. More importantly, recent studies suggest that these astrocytic Ca^{2+} signals are important for the regulation of the arteriole diameter,[33–40] the modulation of the hemodynamic response that generates the intrinsic optical signal,[30] and the control of the blood flow associated with motor behavior.[32] While astrocytic Ca^{2+} signals appear to play important physiological roles as evidenced by the aforementioned studies, they also are activated by pathological neuronal activities, such as during epileptiform discharges,[25,26] and might participate in seizure generation and maintenance, as described later in this chapter.

ASTROCYTES RELEASE CHEMICAL TRANSMITTERS TO MODULATE NEURONAL AND SYNAPTIC FUNCTIONS

The functional consequence of the astrocytic Ca^{2+} signals is the release of gliotransmitters that have been shown to modulate neuronal and synaptic functions [1,2,41](Figure 1B). Several of these gliotransmitters, such as glutamate, adenosine triphosphate (ATP), adenosine, and D-serine, are released in a Ca^{2+}-dependent manner[21,42] through vesicle[43–46] and lysosome exocytosis.[47–49] Astrocytes in culture express the soluble N-ethylmaleimide-sensitive fusion protein receptor (SNARE) complex[43,50–52] which is colocalized with small vesicles positive for vesicular glutamate transporters,[43,50–52] ATP-storing vesicles,[49,53] and D-serine-containing vesicles.[54] Furthermore, ultrastructural studies *in situ* have shown that astrocytic processes contain small synaptic-like vesicles with a diameter of 30 nm, which are located in close proximity to synapses.[43,44] While Ca^{2+}-dependent exocytosis represents the better-characterized pathways of astrocytic release of glutamate, ATP, adenosine, and D-serine, alternative release mechanisms have also been proposed. These mechanisms include reversal of glutamate transporters, connexin/pannexin hemichannels, pore-forming P_2X_7 receptors and swelling-induced activation of volume-regulated anion channels.[55]

In accordance with the concept of the *tripartite synapse* (Figures 1A and B) in which astrocytes are considered to be functionally associated with the pre- and postsynaptic nerve terminals as a third, signaling element at the synapse,[56] it is now well accepted that astrocytes not only respond to neuronal activity but also regulate neuronal excitability, synaptic transmission, and behavior by releasing gliotransmitters.[1,2,14] The mechanisms and consequences of this bidirectional communication between neurons and astrocytes are grouped in a process called *gliotransmission*.[2] The better-characterized gliotransmitters involved in gliotransmission are discussed below.

Release of Glutamate

Glutamate, one of the first gliotransmitters released from astrocytes to be identified, has been reported to exert many effects on synaptic transmission and neuronal excitability. Several studies have shown that glutamate

release from astrocytes through a Ca^{2+}-dependent mechanism activated receptors located at the presynaptic terminals. Through activation of mGluR1[57,58] or *N*-methyl-D-aspartate (NMDA) receptors,[44] astrocytes enhance the frequency of spontaneous and evoked excitatory synaptic currents. Additionally, astrocytic glutamate induces the depression or potentiation[59] of inhibitory synaptic transmission by activation of presynaptic mGluR2/3[60] or kainate receptors,[61] respectively. Moreover, it has been shown that Ca^{2+}-dependent glutamate release from astrocytes at single hippocampal synapses participated in the generation of long-term potentiation (LTP) through the activation of the presynaptic mGluR1.[58] Thus, all of these studies indicate that Ca^{2+}-dependent release of glutamate from astrocytes modulates synaptic strength and plasticity.

Furthermore, various studies have shown that glutamate release from astrocytes activates postsynaptic NMDA receptors to generate slow inward currents (SICs).[62] The observation that spontaneous Ca^{2+} spikes in astrocytes correlates in thalamic neurons with the detection of SICs, suggests that these spontaneous Ca^{2+} signals mediate the release of glutamate from astrocytes to modulate neuronal excitability. Following this observation, multiple experiments performed to determine the nature of these SICs have shown that different protocols used to induce Ca^{2+} signals in astrocytes also increased the occurrence of the SICs.[2,41] For example, ligands that induce astrocytic Ca^{2+} signals,[63–68] photolysis of caged Ca^{2+} that has been selectively loaded into astrocytes,[64,69] single astrocyte depolarization,[70] and electrical stimulation of excitatory presynaptic terminals[64,69] all resulted in Ca^{2+} elevations in astrocytes and occurrence of SICs in nearby neurons. Additionally, SICs have been described in different brain regions[71] such as the thalamus, the cortex, the hippocampus, the nucleus accumbens, the olfactory bulb and, more recently, the brainstem.[72] Typically, SICs are mediated by the activation of a specific group of extrasynaptic NMDA receptors containing the NR2B subunit since they are inhibited by ifenprodil, a selective antagonist of NR2B-NMDA receptors.[64] Furthermore, SICs are insensitive to tetrodotoxin (TTX) and tetanus neurotoxin (TeNT), two substances that block the generation of action potential and the synaptic release of neurotransmitters, respectively,[64] confirming their non-neuronal origin. A recent study also demonstrated that the generation of SICs depends on the duration and kinetics of the Ca^{2+} signals in astrocytes,[68] although Fiacco et al.[73] suggested an alternative interpretation. More importantly, when SICs reach sufficient amplitude, they can trigger burst of action potentials that occur with a high degree of synchronicity in hippocampal pyramidal neurons over short distances of 100 μm.[63,64] Thus, glutamatergic gliotransmission increases neuronal excitability and operates as a non-synaptic mechanism for neuronal synchronization. This form of communication between neurons and astrocytes could represent a significant source of excitation during epileptic discharges as discussed later in this chapter.

Release of D-Serine

The presence of serine racemase, an enzyme required for the conversion of L- to D-serine, in astrocytes has led to the idea that the amino acid might be a significant player in the regulation of the NMDA receptors by acting as the natural substrate for glycine binding sites on the receptor.[74,75] D-Serine-containing vesicles are released from astrocytes in a Ca^{2+}-dependent manner and act as a coagonist of the NMDA receptor.[54] In the supraoptic nucleus of the hypothalamus, the dynamic astrocytic coverage of synapses dependent on physiological signals influences extracellular levels of astrocytic D-serine and consequently leads to a certain form of metaplasticity.[76] For example, the high degree of synaptic coverage by astrocytes seen in virgin rodents induces LTP, whereas the reduced astrocytic coverage of synapses that occurs during lactation induces long-term depression.[76] More recent work has addressed the role of D-serine release in NMDA receptor-dependent LTP in the Schaffer collateral-CA1 synapses.[77] In this work, the selective inhibition of serine racemase in one astrocyte suppressed local LTP induction, demonstrating that one astrocyte is the direct source of D-serine in the hippocampus and can modulate synaptic input plasticity on nearby neurons.

Release of ATP

The release of ATP from astrocytes has been known for a long time and was initially proposed as a mechanism for the propagation of intercellular Ca^{2+} waves through the astrocytic syncytium.[3,11,78] First, ATP is released from astrocytes during Ca^{2+} wave propagation.[11,79] Second, the propagation can be abolished by antagonists of purinergic P_2Y receptors[11,79–81] or the ATP-degrading enzyme apyrase.[11,81] Third, visualization of the release of ATP demonstrates that its velocity correlates with that of the Ca^{2+} wave in astrocytes.[81] These results, suggest that ATP could be an extracellular messenger and a primary signal for the Ca^{2+} wave propagation. While mechanisms of glutamate and D-serine release from astrocytes are experimentally well described, the mechanisms underlying the release of ATP from astrocytes are less well understood. The release of ATP is reduced by inhibitors of several anion channels,[82,83] gap junctions hemichannels,[78,84,85] and pore-forming P_2X_7 receptors,[86] suggesting the involvement of different pathways in the release. Additionally, ATP release from astrocytes is partly Ca^{2+}- and SNARE proteins-dependent.[84,87] Further, astrocytes also display vesicles that contain ATP, and vesicular adenosine triphospatase (ATPase) inhibitors block the release of ATP.[53,87] More importantly, Pascual et al.[88] generated inducible transgenic mice that express a dominant-negative SNARE (dnSNARE) domain of vesicle-associated membrane protein 2 (VAMP2) selectively in astrocytes to suppress the exocytotic release of chemical transmitters from astrocytes. Interestingly, using these transgenic astrocyte-specific dnSNARE mice, it was shown that astrocytes regulate the activation of neuronal A1 receptors that are responsible for presynaptic inhibition of excitatory synaptic transmission in the hippocampus and cortex.[88–90] Bioluminescence imaging demonstrated that this molecular genetic manipulation led to reduced extracellular ATP, and pharmacological evidence was consistent with the notion that astrocyte-derived extracellular ATP is hydrolyzed to adenosine to cause a tonic suppression of synaptic transmission.[88] The mechanism of release of these purines has not been investigated further. The recent discovery of Sawada et al.[91] demonstrated that a novel member of an anion transporter family functioning as a vesicular nucleotide transporter was highly expressed in astrocytes. Together these findings raise the possibility that astrocytes release ATP by exocytosis. However, before such a conclusion is drawn, considerable additional studies will be required.

Once ATP is released from astrocytes, it can exert physiological effects modulating neuronal excitability. For example, in hypothalamic slices, astrocytes express α-1 adrenergic receptors, and in response to adrenergic input they release ATP, which acts on P_2X_7 receptors localized on nearby magnocellular neurosecretory cells (MNCs). As a result, there is an enhancement of AMPA receptor surface expression and an increase in the amplitude of miniature excitatory postsynaptic current in these cells.[92] More recent studies have confirmed postsynaptic effects of ATP on MNCs. Using combined two-photon Ca^{2+} imaging, photolysis of caged compounds and electrophysiology, it has been shown that there is a mGluR1-dependent Ca^{2+} increase in astrocytes along with ATP release resulting in an increase in the amplitude of miniature excitatory postsynaptic currents of MNCs through the activation of postsynaptic P_2X_7 receptors.[93] Additionally, another study from Zhang et al.[94] in hippocampal cultures has shown that the release of ATP from stimulated astrocytes was able to depress glutamatergic neuronal transmission through the direct activation of P_2Y receptors. Interestingly, using hippocampal slices, Zhang et al.[94] also stated that the glutamatergic synaptic depression was due to the ATP metabolite adenosine, which acted on the A1 adenosine receptors. Altogether, these results suggest that ATP release from astrocytes can modulate neuronal excitability and synaptic transmission through direct and indirect actions. The indirect action of astrocytic ATP requires its conversion to adenosine, a metabolite known to have multiple effects on neuronal and synaptic functions, as described below.

Adenosine Derived from Astrocyte-Released ATP

Once ATP is released into the extracellular space, a variety of ectonucleotidases hydrolyze ATP to AMP and then a 5′-nucleotidase converts AMP to adenosine,[95] which is known to be a powerful modulator of synaptic activity via its actions on the G-protein-coupled adenosine receptor subtypes (A1, A2, and A3).[96] In the retina, adenosine derived from astrocyte-released ATP can activate A1 receptors coupled to K^+ channels, which

hyperpolarize neurons and decrease their excitability.[97] In the hippocampus, the adenosine thus produced results in presynaptic inhibition of excitatory synaptic transmission mediated by the activation of A1 receptors.[88,94,98] Moreover, chelating Ca^{2+} in astrocytic syncytium and the use of glial-specific toxins interfere with the A1-dependent synaptic depression, confirming the glial origin of this process.[98] In addition to the mechanisms described above, astrocytes can directly release adenosine, especially in response to hypoxic stimulation[99,100] even though release of adenosine is more typical of neurons.[101] In that case, the release of adenosine from astrocytes depends on export of adenosine through the equilibrative nucleoside transporters.

Recent work using the dnSNARE animals has shown that astrocytic adenosine is important for sleep homeostasis by participating in the accumulation of sleep pressure and contributing to cognitive deficits associated with sleep loss.[90] Moreover, another study has shown that the dnSNARE animals displayed a 50% reduction in surface expression of NMDA receptors, resulting in a decrease in cortical slow oscillations.[89] Thus, these studies identify astrocytes as a major regulator of the activation of neuronal A1 receptors, and thus presumably as a source of adenosine in the brain, and suggest that purinergic gliotransmission plays an important role in synaptic transmission, plasticity, and behavior.

REACTIVE ASTROCYTOSIS AND EPILEPSY

Reactive changes in astrocytes are frequently encountered in the hippocampus in association with temporal lobe epilepsy (TLE) in humans[102] and with animal models of epilepsy.[103,104] These reactive changes, termed *reactive astrocytosis*, generally involve increases in astrocyte size and number[103,104] and often occur together with neuronal loss and synaptic rearrangements.[103,105] Reactive astrocytes exhibit an increased expression of glial cytoskeletal proteins, glial fibrillary acidic protein (GFAP), and vimentin, which are therefore used to assess the development of reactive astrocytosis.[106,107] Interestingly, recent studies have shown that reactive astrocytosis was also accompanied by a loss of the astrocytic domain organization[108] and the generation of new astrocytes from stem cells.[109] More importantly, in addition to morphological changes, many proteins are up- or downregulated in reactive astrocytes, leading to changes in cellular functions.[17] Whether these functional changes modify seizure susceptibility is an intriguing notion receiving increased attention. In the following sections, we discuss recent evidence suggesting that changes in expression of two astrocyte-specific enzymes, glutamine synthetase (GS) and adenosine kinase (ADK), could promote seizures in the epileptic brain.

Reactive Astrocytosis and GS Downregulation in Epilepsy

In the brain, GS is expressed almost exclusively by astrocytes[110] and is responsible for the conversion of synaptically released glutamate into glutamine after the neurotransmitter is taken up by transporters into the synaptically associated glia[111] (Figure 2). This glutamate-glutamine cycle normally serves as a major mechanism for ammonia detoxification in the brain and also as a buffered reservoir of a precursor (glutamine) for glutamate and GABA synthesis[112] (Figure 2).

Early studies performed during epilepsy surgery demonstrated that the accumulation and impaired clearance of hippocampal glutamate in TLE were due to a slowing in the conversion of glutamate to glutamine,[113] suggesting a role for GS deficiency in epilepsy. Later, this observation was supported by two discoveries showing the loss of GS in clinical and experimental epilepsies. First, hippocampal tissue removed from patients with TLE during epilepsy surgery was characterized by downregulation of GS.[114] Second, GS was downregulated with elevated GFAP immunoreactivity during the chronic phase of epilepsy in an animal model of TLE.[115] More recently, an animal model of chronic GS deficiency in the hippocampus has been developed to replicate the situation in human TLE.[116,117] In these studies, freely moving rats monitored with video and electroencephalography developed clusters of spontaneous recurrent seizures and neuropathological changes like those seen in human TLE after a continuous intrahippocampal infusion of the GS inhibitor methionine sulfoximine (MSO).

More importantly, recent studies suggest that downregulation of GS and the consequent reduction in the pool of the GABA precursor glutamine could partially deplete inhibitory synaptic terminals of GABA and impair GABAergic inhibition. In support of this notion, Liang et al.[118] showed that selective blockade of GS by MSO reduced the amplitude of inhibitory postsynaptic currents (IPSCs) in CA1 pyramidal neurons during repetitive stimulus trains. Interestingly, the MSO effect was prevented by the replenishment of glutamine in the bath perfusion.[118] Additionally, recent experiments performed with the same preparation showed no significant effects of MSO on glutamatergic transmission.[119] Together, these results suggest that the glial glutamate-glutamine cycle is the major contributor to synaptic GABA release and regulates inhibitory synaptic strength.

More recently, we tested the hypothesis that GS deficits may be contributing to TLE by reducing synaptic inhibition in the vicinity of reactive astrocytes.[120] First, in this study, a higher-titer viral transduction of astrocytes with enhanced green fluorescent protein (eGFP) via bilateral injections of adeno-associated virus into the mice hippocampus led to reactive astrocytosis. Reactive eGFP-astrocytes showed high levels of expression of GFAP and vimentin, while nearby neurons and microglia were not altered. Second, consistent with the studies suggesting that reduced GS expression levels are associated with the development of astrocytosis,[111,114,115] a pronounced downregulation of GS associated with enhanced GFAP and vimentin expression was observed. Third, we hypothesized that the GS downregulation and, therefore, the reduced glutamine could generate a deficit in the inhibitory synaptic transmission in neurons located in the eGFP-positive areas. To test this hypothesis, we used brain slices to record evoked IPSCs (eIPSCs) and spontaneous miniature IPSCs (mIPSCs) in CA1 pyramidal neurons located in the eGFP-positive areas from mice treated with the adeno-associated virus. As expected, the amplitude of eIPSCs and the amplitude/frequency of mIPSCs were reduced while evoked excitatory postsynaptic currents (eEPSCs) were not altered, suggesting that only inhibitory synaptic transmission was impaired in CA1 pyramidal neurons proximal to reactive astrocytes. Furthermore, MSO had no effect on the amplitude of eIPSCs in CA1 neurons located in the vicinity of eGFP-positive reactive astrocytes, confirming that the evoked failure was due to GS deficiency. Interestingly, bath application of glutamine increased the amplitude of eIPSCs, providing further proof that eIPSCs failure was mediated by neuronal glutamine starvation. These results suggest that reactive astrocytosis-induced GS downregulation leads to an interrupted neuronal glutamine supply, impaired neuronal production of GABA, and compromised inhibitory synaptic transmission (Figure 2). Finally, to determine whether reactive astrocytosis was associated with network hyperexcitability, we used voltage-sensitive dye imaging techniques and stimulation of the temporoammonic pathway between the entorhinal cortex and CA1, a particular technique of stimulation known to constrain excitatory postsynaptic potentials (EPSPs) by triggering a feedforward inhibition. In eGFP-positive hippocampal slices, the EPSPs propagated much further than in control slices from untreated animals. Furthermore, bath perfusion of eGFP-positive hippocampal slices with exogenous glutamine reduced the areas activated by the temporoammonic pathway stimulation. Thus, inhibitory deficits associated with reactive astrocytosis lead to hyperexcitability of the hippocampal network and this can be prevented by exogenously supplied glutamine. Altogether, these results suggest that the GS loss seen during reactive astrocytosis could contribute to elevated seizure susceptibility in TLE by reducing neuronal inhibition (Figure 2). Consequently, protecting GS function might represent a promising therapeutical strategy to prevent seizures.

Reactive Astrocytosis and ADK Upregulation in Epilepsy

Under physiological conditions extra- and intracellular levels of adenosine are rapidly equilibrated via distinct families of nucleoside transporters.[121] Intracellularly, adenosine is rapidly metabolized by phosphorylation to AMP via ADK,[121] the key enzyme of adenosine metabolism,[122] which, in adult brain, is predominantly expressed in astrocytes.[123] Thus, ADK is ideally located to control the astrocyte-based adenosine cycle by driving the influx of adenosine into the cell via bidirectional nucleoside transporters[122] (Figure 3). Adenosine, in particular, plays a prominent role in seizure regulation and has been found to be elevated in patients following seizures, leading to the conclusion that adenosine released during a seizure mediates seizure arrest and postictal refractoriness.[124] The adenosine A1 receptor-mediated functions are largely responsible for the anti-convulsant

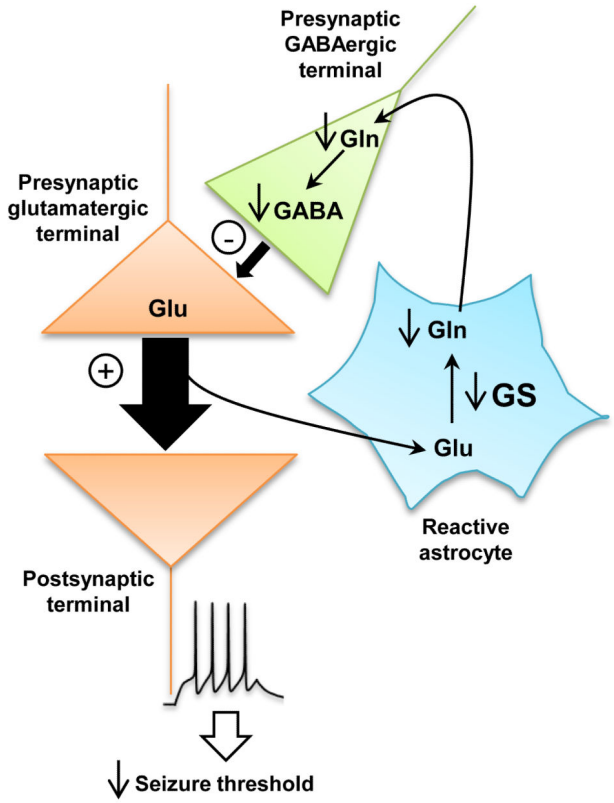

Figure 2. The activity change of glutamine synthetase (GS) in reactive astrocytes contributes to seizure development. Glutamate (Glu) released at the excitatory synapse is converted into glutamine (Gln) through activity of GS in astrocytes. Glutamine is used as a precursor for synthesis of GABA in GABAergic neurons. The loss of GS in reactive astrocytes leads to a decrease in Gln and GABA levels in GABAergic terminals. Consequently, presynaptic GABAergic inhibition is reduced, increasing presynaptic release of Glu. In turn, Glu enhances the excitability of postsynaptic neurons, leading to a reduction in the seizure threshold.

and neuroprotective activity of adenosine.[96] Thus, binding of adenosine to A1 receptors that are highly expressed in the hippocampus, leads to decreased neuronal excitability and synaptic transmission through postsynaptic membrane hyperpolarization and inhibition of presynaptic release, respectively[96] (Figure 3). Consequently, changes in the homeostasis of the astrocyte-based adenosine cycle are to be expected in the epileptic brain.

Recent studies from Boison's group have identified ADK as molecular link between reactive astrocytosis and neuronal dysfunction in epilepsy leading to the *ADK hypothesis of epileptogenesis*.[122] Thus, transgenic mice overexpressing ADK in the brain displayed a reduced tone of the endogenous anti-convulsant adenosine, leading to the emergence of spontaneous chronic seizures.[125] Furthermore, a direct association between the development of reactive astrocytosis and the upregulation of ADK has been shown.[125,126] Conversely, transgenic mice with a forebrain-selective reduction of ADK were resistant to seizure development.[126] Together, these studies indicate that reactive astrocytosis causes overexpression of ADK, which was shown to be sufficient to trigger seizures (Figure 3). Thus, reconstitution or augmentation of adenosine and/or inhibition of ADK constitutes a pharmacological rationale for seizure suppression. In support of this notion, it has been demonstrated that intrahippocampal implants of ADK-deficient stem cell-derived neuronal precursors suppress kindling epileptogenesis, suggesting that any therapy leading to focal augmentation of the adenosine system has the potential to prevent epileptogenesis.[121,127,128]

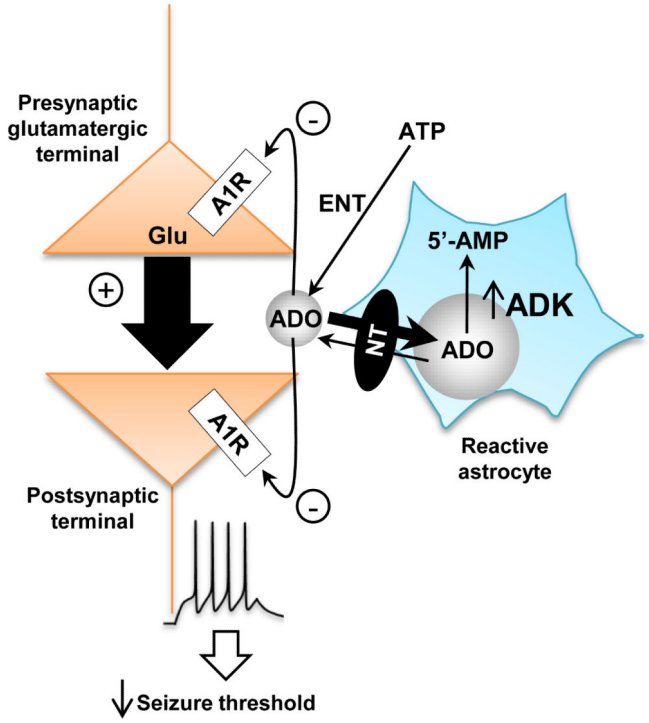

Figure 3. The activity change of adenosine kinase (ADK) in reactive astrocytes contributes to seizure development. Extracellular ATP is rapidly degraded into adenosine (ADO) by ectonucleotidases (ENT). Adenosine exerts both pre- and postsynaptic inhibitory effects via adenosine A1 receptors (A1R). The extra- and intracellular levels of ADO are equilibrated via nucleoside transporters (NT). The intracellular level of ADO depends on the activity of ADK in astrocytes that converts ADO into 5′-adenosine-monophosphate (5′-AMP). Overexpression of ADK in reactive astrocytes increases the influx of ADO into the cell, decreasing extracellular levels of ADO. Consequently, pre- and postsynaptic inhibitory effects of ADO are reduced leading to a decrease in seizure threshold.

ASTROCYTIC Ca^{2+} SIGNALS, GLUTAMATE RELEASE AND EPILEPSY

Several lines of evidence indicate that Ca^{2+} elevations in astrocytes leading to glutamate release and synchronization of neuronal firing (see above) could be involved in epilepsy. Thus, below we discuss recent studies supporting the potential role for astrocytic Ca^{2+} signals and glutamate release in the generation of epileptiform activity.

Astrocytic Ca^{2+} Signals and Epilepsy

Under physiological conditions, Ca^{2+} signals in astrocytes arise to activation of mGluRs (see above). Interestingly, it has been shown that the protein expression levels of mGluRs were increased in reactive astrocytes in animal models of epilepsy[129,130] and in hippocampal specimen from patients with TLE.[131] Furthermore, hippocampal cultured astrocytes derived from patients with TLE show increases in Ca^{2+} signals.[111] More recently, studies have shown that cortical epileptiform activity induced *in vivo* in anesthetized mice with bicuculline,[25] picrotoxin,[26] or the A-type K^+ channel blocker 4-aminopyridine[132] (4-AP) were associated with increases in Ca^{2+} signals in astrocytes, which could be suppressed by intraperitoneal injections of several anti-epileptic drugs including valproate, gabapentin and phenytoin.[132] This positive correlation between increased astrocytic Ca^{2+} signaling and epileptiform activity onset suggests that these Ca^{2+} signals might contribute to seizure generation.

Astrocytic Glutamate Release and Epilepsy

Studies performed by Kang et al.[66] first demonstrated that Ca^{2+}-dependent release of glutamate from astrocytes might be involved in the generation of epileptiform discharges. In brain slices, the infusion of IP_3 through a patch-clamp pipette to increase Ca^{2+} signals into astrocytes was followed by the occurrence of slow, decayed transient inward currents (STCs) in nearby CA1 pyramidal neurons.[66] In current-clamp, STCs were able to depolarize the neuronal membrane and trigger firing of action potentials. This neuronal depolarization was reminiscent of the paroxysmal depolarization shift (PDS) underlying an interictal epileptiform event[133] that is known to be synchronized over many millimeters of epileptic brain.[134]

Later work from the same group supports the role of astrocytes in generating epilepsy.[132] Using 4-AP to induce interictal epileptiform activity in hippocampal slices, Tian et al.[132] showed that PDSs persisted in the presence of both TTX and different Ca^{2+} channel blockers that suppressed presynaptic release but were blocked by the ionotropic glutamate receptor antagonists CNQX and D-AP5. Thus, these results suggest that PDSs were triggered by release of glutamate from extrasynaptic sources. To determine whether Ca^{2+} signals in astrocytes are associated with PDSs, the authors used photolysis of caged Ca^{2+}. However, photolysis of caged Ca^{2+} in one astrocyte induced local PDS in the presence of TTX, suggesting that Ca^{2+} elevations in astrocytes lead to glutamate release, which targets nearby neurons to generate PDSs, the hallmark of epileptic activity.[133]

In contrast to studies from Nedergaard's group described above, our work[135] suggested that glutamate release from astrocytes was not necessary for the generation of epileptiform activity but rather that it could be modulatory. In this work, we induced both ictal and interictal epileptiform activity in hippocampal slices by removing Mg^{2+} (0 Mg^{2+}) in the presence of picrotoxin. The epileptiform activity thus generated triggered Ca^{2+} signals in astrocytes and increased the frequency of NMDA-receptor-mediated SICs that share certain properties with STCs. However, when slices were pre-incubated with D-AP5 to block NMDA-receptor mediated SICs, treatment of the slices with 0 Mg^{2+} and picrotoxin was still able to trigger epileptiform activity, suggesting that glutamate release from astrocytes and SICs *per se* were not required to initiate epileptiform activity. Interestingly, D-AP5 reversibly reduced the duration of both ictal and interictal epileptiform events, suggesting that astrocytic glutamate, even though it is not necessary for the generation of epileptiform activity, might be important in determining the strength of epileptiform discharges.[135]

Faced with these two conflicting studies,[132,135] recent work by Gomez-Gonzalo et al.[65] attempted to determine the role of astrocytes in the generation of focal ictal discharges. First, the authors showed in enthorinal cortex (EC) slices from rats that while 0 Mg^{2+}/picrotoxin induced both ictal and interictal discharges, only ictal discharges were associated with Ca^{2+} elevations in astrocytes. Second, the frequency and duration of ictal discharges, as well as their associated astrocytic Ca^{2+} signals, were attenuated by mGluR5 and P_2Y receptor antagonists, whereas interictal discharges were unaffected, indicating that astrocytic Ca^{2+} elevations mediated by mGluR5 and P_2Y receptors do not have a role in the generation of interictal discharges. Furthermore, the selective stimulation of astrocytes with the peptide Thr-Phe-Leu-Leu-Arg-NH_2 (TFLLR-NH_2), known to induce astrocytic glutamate release and SICs via activation of the PAR-1 thrombin receptor,[68,136] was able to generate ictal discharges in the presence of 0 Mg^{2+}/picrotoxin, suggesting that astrocytic glutamate release was sufficient to initiate ictal discharges in EC slices prone to generate epileptiform activity. Through a series of elegant experimental manipulations, Gomez-Gonzalo et al. were able to show that while neuronal activity is critical for the generation of ictal discharges, astrocytes can modulate the threshold for the generation of this epileptiform activity.[65]

In addition to its potential role in the generation of epileptiform activity, our recent study suggested that Ca^{2+}-dependent release of glutamate from astrocytes could also contribute to the typical delayed neuronal death observed after status epilepticus (SE).[137] Using two-photon *in vivo* microscopy and the pilocarpine model of epilepsy to induce SE in mice, we have shown that SE enhanced Ca^{2+} signals in astrocytes for 3 days and that this enhancement was associated with the period of delayed neuronal death.[137] More importantly, we found that

post-SE administration of MPEP to block mGluR5 mediated Ca^{2+} signals in astrocytes (see above) and ifenprodil to selectively block NMDA-NR2B receptors mediated SICs (see above) provided significant neuronal protection.[137] Furthermore, we have shown that selective loading of Ca^{2+} chelators into astrocytes after SE also led to neuronal protection, suggesting neurotoxic roles for glutamatergic gliotransmission in epilepsy.[137] This notion is also supported by previous studies demonstrating that the activation of extrasynaptic NMDAR-NR2B receptors stimulates cyclic adenosine monophosphate response element binding protein (CREB) dephosphorylation and neuronal death.[138]

While controversial observations exist concerning the involvement of astrocytes in the generation of seizures it is clear that astrocytic Ca^{2+} signals and astrocytic glutamate play an important role in the mechanisms of epilepsy. However, we now await the introduction of astrocyte selective inhibitors to define the role of astrocytes in the process. Thus, gliotransmission should be considered as a potential therapeutic target in epilepsy.

CONCLUDING REMARKS

The extraordinary evolution of *in vivo* Ca^{2+} imaging techniques allowed us to appreciate the highly dynamic nature of Ca^{2+} signaling in astrocytes under physiological conditions. This Ca^{2+} excitability represents an original pathway for astrocytes to integrate and process the neuronal information in the brain. In response to neuronal activity, astrocytes release gliotransmitters to modulate both excitatory and inhibitory synaptic transmission, and consequently affect brain plasticity and mammalian behavior. It is striking to see how astrocytes react to epilepsy by changing their shape and functions. This glial reactivity leads to increases in neuronal excitability and consequently accelerates the evolution of this neuronal disorder. In addition to astrocytic reactivity, extensive experimental research suggests that astrocytic Ca^{2+} signaling and gliotransmitter release participate in the generation of seizures. However, data coming from this research are currently controversial mainly due to the different experimental approaches and lack of selective pharmacological tools to block astrocytic Ca^{2+} signaling and gliotransmission. In the future, the development of transgenic animals bearing specific deficiencies interfering with astrocytic Ca^{2+} signals and gliotransmission and the use of chronic models of epilepsy that more closely mimic the complex feature of seizures in epileptic patients will represent new approaches to identify the role of astrocytes in the generation of seizures and rigorously evaluate the idea that these glial cells represent a target for developing new therapeutic strategies for epilepsy.

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DISCLOSURE STATEMENT

The authors declare no conflicts of interest.

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