Sorting Out Astrocyte Physiology from Pharmacology

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Key Words

Gq GPCR, glia, IP_3R, Ca^{2+}, glutamate, microdomain

Abstract

A number of exciting findings have been made in astrocytes during the past 15 years that have led many researchers to redefine how the brain works. Astrocytes are now widely regarded as cells that propagate Ca^{2+} over long distances in response to stimulation, and, similar to neurons, release transmitters (called gliotransmitters) in a Ca^{2+}-dependent manner to modulate a host of important brain functions. Although these discoveries have been very exciting, it is essential to place them in the proper context of the approaches used to obtain them to determine their relevance to brain physiology. This review revisits the key observations made in astrocytes that greatly impact how they are thought to regulate brain function, including the existence of widespread propagating intercellular Ca^{2+} waves, data suggesting that astrocytes signal to neurons through Ca^{2+}-dependent release of glutamate, and evidence for the presence of vesicular machinery for the regulated exocytosis of gliotransmitters.

INTRODUCTION

Astroglia: describes astrocytes in culture to help clarify studies done on cultured astrocytes versus astrocytes in situ or in vivo

Microdomain: a region of astrocyte processes exhibiting a Ca^{2+} elevation independent from the rest of the cell

Spontaneous: activity that occurs in the absence of user-induced stimulation. This activity may be initiated from external signals or be intrinsic to the cell

The role of astrocytes in acute brain signaling is a rapidly growing field of neuroscience now receiving considerable attention (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org** to read "What Is an Astrocyte?"). Astrocyte Ca^{2+} elevations, which are considered the primary form of astrocyte excitability (1), have been reported to significantly modulate or drive essential brain activities, including neurite outgrowth during development (2), neuronal synaptic transmission (3–6), long-term potentiation (LTP) (7–9), heterosynaptic depression (10–12), presynaptic release probability (7, 9, 13) and coupling cerebral blood flow to active neurons (14). In all of these cases, Ca^{2+}-dependent release of gliotransmitters from astrocytes is the reported mechanism driving the astrocyte-mediated effects. The significance of these findings suggests that in the absence of astrocyte Ca^{2+} elevations, either intrinsically or in response to neuronal activity, brain function and behavior should be severely impaired.

Our understanding of calcium-dependent gliotransmitter release from astrocytes is at a crossroads often faced by growing areas of neuroscience. In 2003, Lisman et al. (15) discussed the perils and progress of LTP, and many of the questions raised are entirely applicable to the effects attributed to astrocyte Ca^{2+}: Are astrocytes (analogous to specific molecules in LTP) implicated on the basis of correlative evidence, i.e., found in the right place at the right time, without solid experimental proof of their involvement? Do astrocyte-mediated effects occur only under highly specific conditions? What of a primary preparation in the field, the tissue slice, which undergoes important metabolic and structural changes during preparation and continues to change during an experiment? Do we have the necessary tools to resolve an astrocyte-mediated effect from effects produced by direct activation of neurons, analogous to the difficulty resolving pre- versus postsynaptic effects in LTP? Finally, and most importantly, do the effects we measure in our preparations occur in vivo during normal behavior and serve a biological purpose? In this review, we aim to analyze the underlying properties of astrocytes at the epicenter of the astrocyte-linked phenomena: astrocyte Ca^{2+}, astrocytic vesicles, and astrocytic Ca^{2+}-dependent release of glutamate, in the context of the approaches and conditions used to acquire the data.

WIDESPREAD PROPAGATING ASTROCYTE CA^{2+} WAVES: FACT OR ARTIFACT?

Stimulating a single astroglial cell in a culture dish results in an intracellular Ca^{2+} elevation that rapidly propagates throughout many surrounding cells in the confluent monolayer (16, 17). The original report of this discovery by Cornell-Bell et al. (18) garnered much attention because it suggested that networks of astrocytes may constitute a long-range signaling system within the brain. However, other cultured cell types, such as C6 glioma cells (19), airway epithelial cells (20), primary mouse cortical neurons and GT1–1 immortalized neurons (21), rat hepatic Hep-G2 cells (22), rat medial collateral ligament cells (23), and human osteoblast-like cells (24), also propagate Ca^{2+} intercellularly over long distances. Therefore, this phenomenon appears to be a general feature of cultured cells (25) rather than a special property of cultured astroglia.

Although readily observed in cultured cells, there is very little evidence for long-range propagating intercellular astrocyte Ca^{2+} waves in intact cortical tissue during basal levels of neuronal activity. Under basal conditions, most astrocyte Ca^{2+} elevations in situ and in vivo appear to be predominantly localized to small territories of astrocyte processes. Bergmann glia in the cerebellum (26) and astrocytes in the hippocampus (27) exhibit microdomain spontaneous Ca^{2+} activity whereby localized Ca^{2+} elevations occur within isolated compartments. In the cerebellum, local astrocyte Ca^{2+} increases occur during low-level stimulation of granule cell parallel fibers (**Figure 1**),

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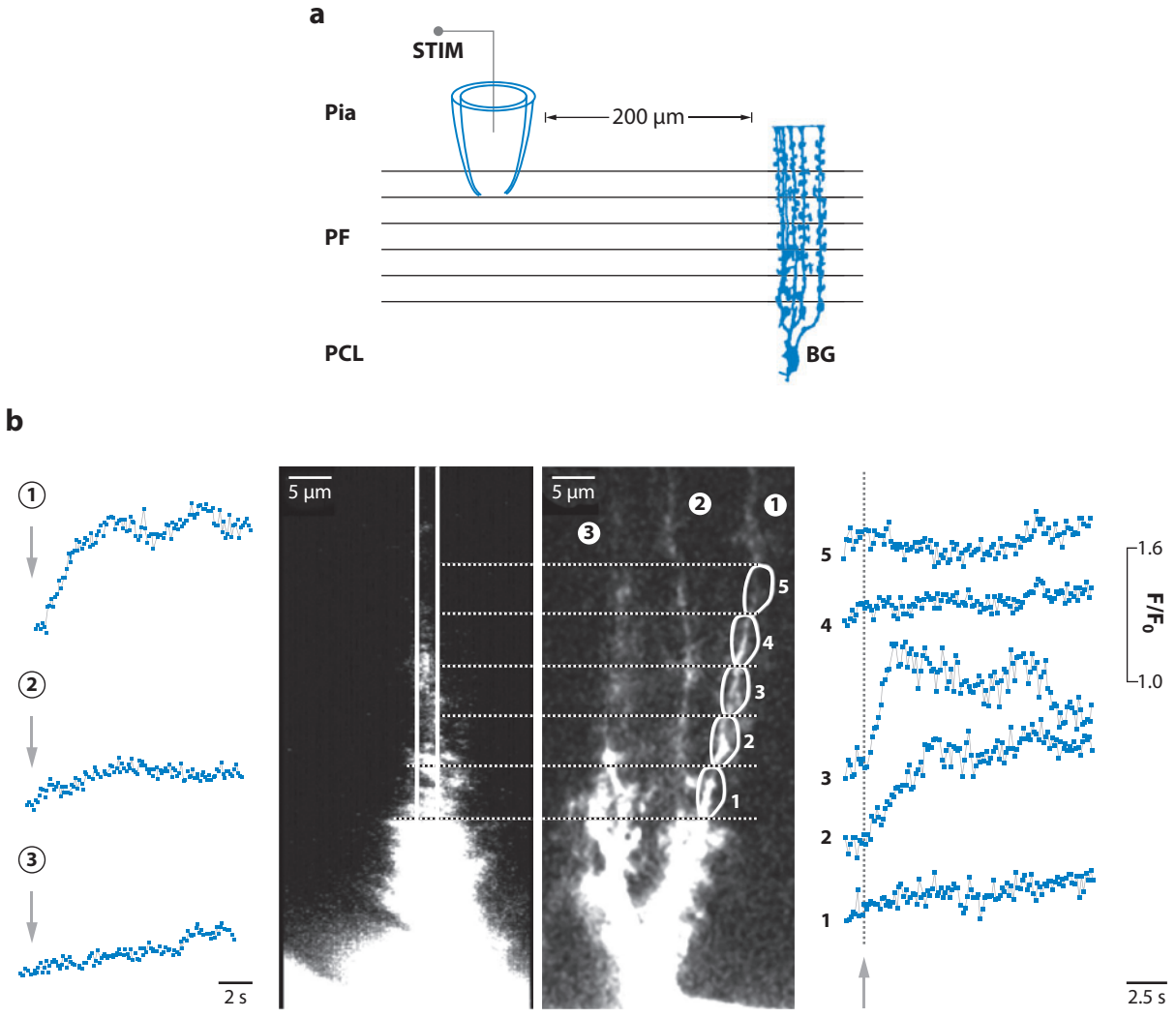

Figure 1

Stimulation of parallel fibers triggers local calcium signals in Bergmann glial cells. (*a*) Experimental protocol. Parallel fibers (PF) were stimulated via a pipette connected to a stimulator (STIM) and Ca^{2+}-dependent fluorescence responses were recorded in a Bergmann glial cell (BG); PCL, Purkinje cell layer. (*b*) Confocal fluorescence intensity image of a patch-clamped Bergmann glial cell dialyzed with the calcium indicator, Oregon green 488 BAPTA-1 (*right*). Three processes were distinguished (indicated as 1–3). Calcium signals in response to PF stimulation were measured independently for each process (*left*). Time of PF stimulation is marked by arrows. The responding process (1) was then further subdivided into five regions of interest, in which calcium signals were measured separately (*right*). Time of PF stimulation is marked by an arrow and a dotted line. The left image was obtained from a sequence of serial sections and shows the cell in depth (turned by 90° as compared with the right image). The lines indicate the focal plane used for $[Ca^{2+}]_i$ recordings. The process is thus within the volume from which recordings were obtained. Reprinted with permission from Nature Publishing Group, copyright 1999 (26).

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suggesting that spontaneous microdomain Ca^{2+} oscillations observed in the absence of stimulation are driven by basal granule cell activity (26). In the hippocampus, spontaneous astrocyte Ca^{2+} oscillations remain unchanged during block of vesicular neurotransmitter release, suggesting that this activity is intrinsic to the astrocyte (27). It is difficult to imagine a physiological scenario in which Ca^{2+} waves propagate over hundreds of microns through many astrocytes, given the existence of microdomain Ca^{2+} increases that predominate during basal levels of neuronal activity.

Intrinsic: a type of spontaneous activity that is initiated from within the cell

When synchronized Ca^{2+} elevations are observed between neighboring astrocytes, active neuronal networks are largely responsible, not waves of Ca^{2+} propagating between astrocytes. Spontaneous astrocyte Ca^{2+} elevations become significantly decorrelated (random) during block of neuronal synaptic transmission (28, 29) (**Figure 2**). However, a few small clusters of two to three

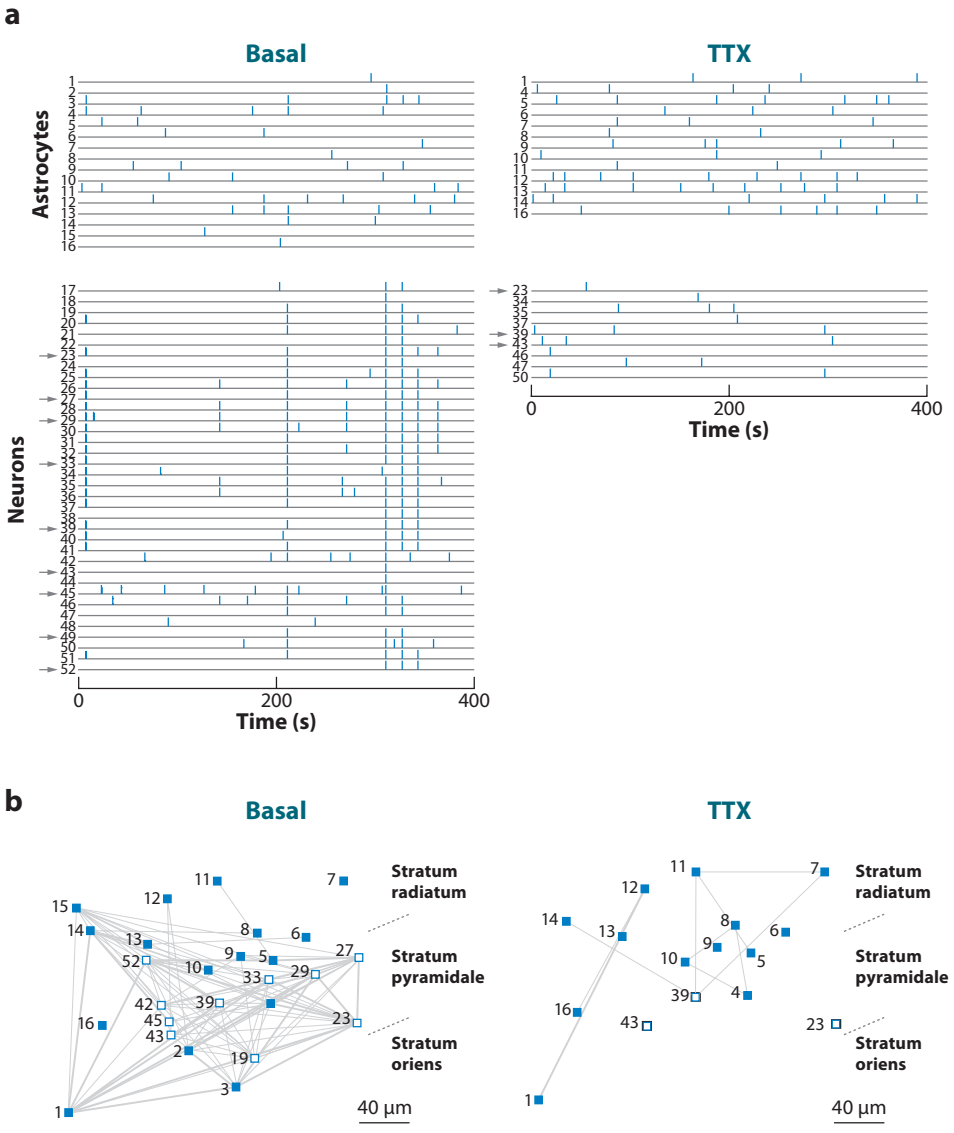

Figure 2

(*Continued*)

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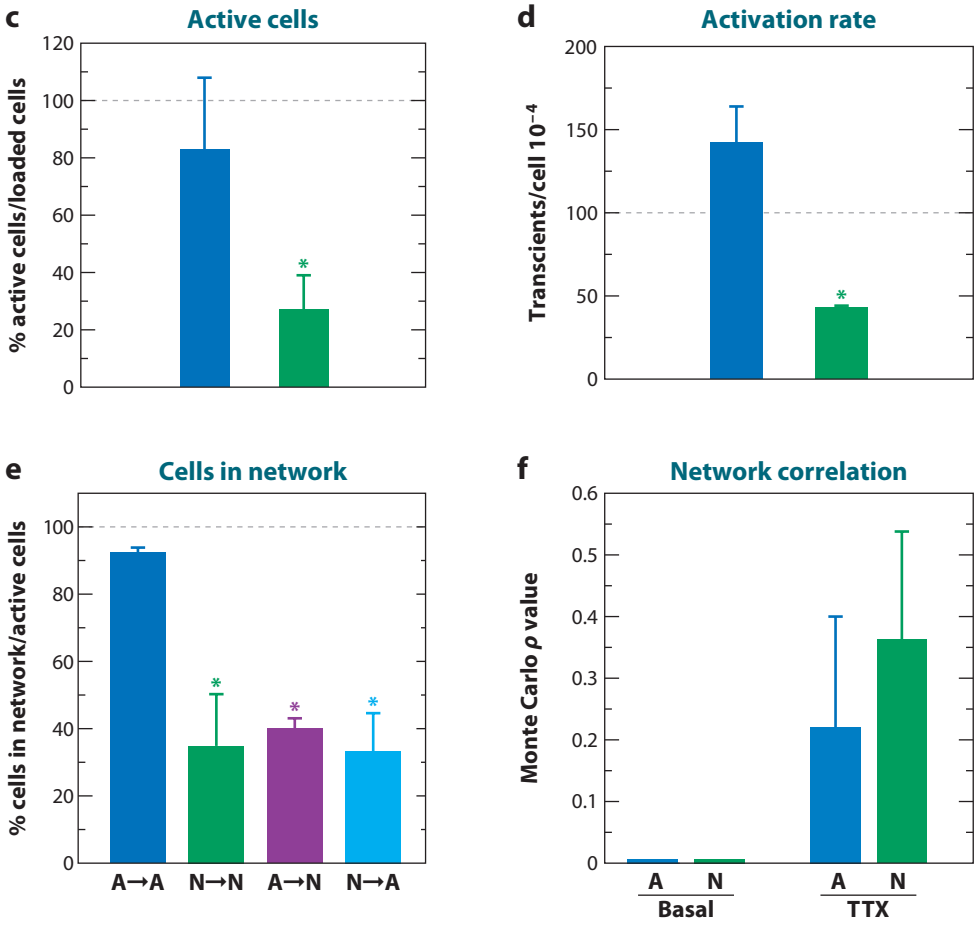

Figure 2

Spontaneous astrocytic correlated network activity is impaired after tetrodotoxin (TTX) treatment. (*a*) Raster plot illustrating the activation profiles of astrocytes (cells *1–16*) and neurons (cells *17–52*) of a hippocampal CA1 field from a P6 glial fibrillary acidic protein-green fluorescent protein (GFAP-GFP) mouse before (basal) and after TTX administration. Although major changes are not observed in the astrocyte population, neuron activity is greatly impaired. (*b*) Correlation maps of all active astrocytes (*blue squares*) and a representative fraction of active neurons (*white squares*, *arrows in raster plots*) illustrated in (*a*). After TTX treatment, active neurons and their correlations are almost absent, whereas correlated astroglial activity persist. (*c*) Spontaneous active astrocytes (*blue bar*) and neurons (*green bar*) in relation to basal conditions after TTX administration. (*d*) Activity rate in astrocytes (*blue bar*) and neurons (*green bar*) in relation to control after TTX administration. (*e*) Histograms summarizing the proportion of spontaneous active cells with statistically significant correlation coefficients after TTX: among astrocytes ($A \rightarrow A$), among neurons ($N \rightarrow N$), percentage of astrocytes coactive with neurons ($A \rightarrow N$), and percentage of neurons coactive with astrocytes ($N \rightarrow A$). (*f*) Average of Monte Carlo *p* values showing the probability that the number of times that any two cells had simultaneous onset of activation was caused by chance. Both astrocytes (*A*) and neurons (*N*) exhibit very significant values in basal conditions, whereas TTX treatment decorrelates spontaneous activity in both neural populations. Significant reductions ($^*p < 0.05$) are observed after TTX treatment. Scale bar, 40 μm. *sr*, stratum radiatum; *sp*, stratum pyramidale; *so*, stratum oriens. Reprinted with permission from The Society for Neuroscience, copyright 2002 (29).

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synchronized astrocytes remain (**Figure 2*b***), suggesting that sometimes Ca^{2+} waves might occur between astrocyte neighbors in the absence of neuronal activity. We have found in our own experiments that robust intracellular Ca^{2+} waves evoked by uncaging inositol triphosphate (IP_3) in single hippocampal astrocytes in P11–16 acute hippocampal slices do not propagate into any neighboring astrocytes (13). It is difficult to reconcile these findings with those of Sul et al. (30), who reported that uncaging glutamate over single hippocampal astrocytes results in global Ca^{2+} elevations that propagate into approximately four neighboring astrocytes. One possible explanation is that Sul and coworkers used P7–12 hippocampal slices versus the P11–16 slices used in our experiments. It is possible that Ca^{2+}-coupled astrocytes may be more prevalent at earlier stages of development (see also 31). Another important consideration is that uncaging glutamate results in stimulation of astrocytic metabotropic glutamate receptor (mGluR) signaling cascades, compared with uncaging IP_3, which results in direct release of Ca^{2+} from intracellular stores. The cascade of Gq G protein–coupled receptor (Gq GPCR) signaling molecules activated following glutamate uncaging may work in concert to mediate Ca^{2+} wave propagation between astrocytes. This explanation does not fit, however, with the many findings showing that uncaging Ca^{2+} or IP_3 in single astrocytes produces robust gliotransmitter release, whereas stimulation of particular Gq GPCRs fails to do so (see below).

Metabotropic glutamate receptor (mGluR): Group 1 mGluRs are Gq GPCRs coupled to the generation of IP_3 and release of Ca^{2+} from the endoplasmic reticulum

Tetrodotoxin (TTX): a sodium channel blocker used to block neuronal action potentials

Overall data suggest that Ca^{2+} might, on rare occasions, propagate between astrocytes in specific circumstances, and on a much smaller scale than that observed between cultured cells. The specific physiological conditions that dictate whether an astrocyte Ca^{2+} response will propagate intercellularly are not known. More work needs to be done in mature tissue to explore the nature of microdomain, global, and propagating astrocyte Ca^{2+} elevations and their physiological importance. The different astrocyte Ca^{2+} increases might each function in a specific way to modulate brain activity. For example, microdomain intrinsic astrocyte Ca^{2+} activity may serve a housekeeping function for the maintenance of astrocyte or neuronal signaling systems, analogous to miniature postsynaptic currents in neurons for the maintenance of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (32). Astrocyte Ca^{2+} increases evoked by neuronal activity might regulate cerebral blood flow to meet energy demands (33). How might local astrocyte Ca^{2+} increases modulate the activity at single neuronal synapses? This level of potential neuronal modulation by astrocytes remains to be explored.

DO ASTROCYTES RESPOND WITH CA^{2+} INCREASES TO BASAL LEVELS OF NEURONAL ACTIVITY?

Astrocytes possess a vast assortment of G protein–coupled receptors (GPCRs) of the Gi, Gs, and Gq families that can be activated pharmacologically but whose function has remained elusive. Porter & McCarthy (34) were the first to demonstrate in acute hippocampal slices that astrocytes in stratum radiatum respond to Schaffer collateral stimulation with Ca^{2+} increases. The astrocytic mGluR-mediated Ca^{2+} responses were elicited only during intense levels of neuronal stimulation. Can astrocytes respond to more physiological levels of neuronal activity? Grosche et al. (26) demonstrated in a very nice study that Ca^{2+} activity is confined to processes of Bergmann glia in the cerebellum following a brief stimulation (10 pulses at 50 Hz) of parallel fibers. Blockade of neuronal activity using tetrodotoxin (TTX) completely eliminated the local glial Ca^{2+} changes. Bergmann glia are very large, specialized astrocytes that receive input from parallel and climbing fibers directly onto Ca^{2+}-permeable AMPA receptors at specialized synoptic structures called ectopic release sites (35, 36). In contrast, astrocytes in cortex and hippocampus are thought to be activated following spillover of glutamate from the synaptic cleft. Therefore, exploring the relationship between low levels of neuronal stimulation and astrocyte Ca^{2+} activity in cortical regions might reveal new information about fundamental neuron-to-glia signaling properties.

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Work out of Craig Jahr's laboratory suggests that astrocyte glutamate transporters sense glutamate released from single neuronal action potentials (C. Jahr, personal communication), indicating the potential for astrocytic mGluR-mediated responses during basal levels of neuronal glutamate release. Protocols designed to study astrocyte Ca^{2+} responses to neuronal activity might include a range of physiological levels of neuronal stimulation to mimic naturally occurring patterns of neuronal bursting, similar to theta burst LTP protocols designed to mimic naturally occurring theta oscillations (37). It is possible that Ca^{2+} responses in cortical or hippocampal astrocytes are not generated by single neuronal action potentials, but occur instead during physiological levels of neuronal bursting activity. Astrocyte Ca^{2+} responses might scale to match the frequency of neuronal transmitter release, or be all-or-none once the threshold for activation of astrocytic Ca^{2+} signaling has been reached.

Recently, Maiken Nedergaard's group demonstrated astrocytic Ca^{2+} signaling evoked by whisker stimulation of mice in vivo (38). Their study demonstrated a low basal Ca^{2+} activity of astrocytes in barrel cortex, and that intense sensory input evoked astrocyte Ca^{2+} increases. Evoked Ca^{2+} elevations started in the astrocyte processes, but eventually propagated throughout the astrocyte and lasted 20 s, on average. Because of a 3 s delay between whisker stimulation and the onset of the astrocyte Ca^{2+} increases, the data suggested that the astrocytes were responding to glutamate spillover from the cortical synapses. In the absence of stimulation, spontaneous Ca^{2+} elevations in astrocyte cell bodies were rare, whereas spontaneous Ca^{2+} activity in astrocyte processes was much more frequent and dynamic. These findings suggest that (*a*) microdomains of Ca^{2+} activity predominate in astrocytes in vivo during basal levels of neuronal activity (discussed above), and (*b*) intense levels of neuronal activity evoke global astrocyte Ca^{2+} elevations.

THE APPROACHES USED TO STIMULATE ASTROCYTE Ca^{2+} ELEVATIONS: AN OVERVIEW

Correlating spontaneous astrocyte Ca^{2+} elevations to changes in a specific neural activity is indirect, leaving open the possibility that astrocytes are simply in the right place at the right time. To provide a stronger link between astrocyte Ca^{2+} elevations and an effect on brain activity, many experimenters have evoked astrocyte Ca^{2+} elevations. Until recently, this was done in one of four ways: (*a*) uncaging caged Ca^{2+} or caged IP_3 directly in the astrocyte, (*b*) mechanical stimulation of the cell with a glass tip, (*c*) electrical stimulation via patch pipette using strong depolarizing stimuli, or (*d*) bath application of agonists to Gq GPCRs coupled to internal Ca^{2+} stores. In all of these approaches, the evoked Ca^{2+} elevations tend to be much more global and propagate with greater speed within single and multiple astrocytes relative to the spontaneous and intrinsic Ca^{2+} activity that, as discussed previously, occurs in the absence of stimulation. There are important additional caveats specific to each approach. For example, uncaging second messengers bypasses intracellular signaling cascades that are activated when Gq GPCRs are stimulated. This plethora of signaling molecules may work together in a tightly coordinated manner to regulate release of gliotransmitters and other astrocytic responses. Mechanical stimulation does not remotely approximate endogenous mechanisms of astrocytic receptor activation or intrinsic Ca^{2+} activity.

Finally, bath application of agonists to endogenous receptors leads to widespread astrocyte Ca^{2+} elevations; however, interpretation of the data is clouded by direct stimulation of neuronal receptors by the applied agonist. Unfortunately, there has often been an astrocentric view to the interpretation of the data, whereby measured effects are attributed to astrocyte receptor stimulation with insufficient consideration given to the certain direct consequences of activating neuronal signaling cascades. One of the most prevalently used agonists to stimulate astrocytes, the group 1 mGluR agonist dihydroxyphenylglycine (DHPG), is a peculiar choice given the many reported direct actions on neurons, including neuronal Ca^{2+} elevations (39, 40); chemically induced

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long-term depression (LTD), a form of synaptic plasticity defined by a weakening of synaptic strength, and subsequent enhancement of LTP (41); long-term depolarization leading to increased neuronal firing (39, 42); and potentiation of N-methyl-D-aspartate (NMDA) receptor currents (43). Yet researchers continue to use DHPG with the intention of selectively stimulating astrocyte Ca^{2+}, with little discussion of the possibility that the effects observed may be due in part to DHPG stimulation of neuronal mGluRs (6). Unfortunately, DHPG is not unique in this regard. Agonists to endogenous Gq GPCRs in brain will generally produce direct actions in neurons that have nothing to do with astrocytes (and vice versa). In fact, we cannot list a single agonist to an identified endogenous Gq GPCR in the brain that is known with certainty to activate only astrocyte signaling. Conclusions drawn from experiments using any of the traditional approaches must be considered cautiously.

Dihydroxyphenylglycine (DHPG): a selective group 1 mGluR agonist

MrgA1R: a receptor belonging to the Mas related gene family that is endogenously expressed by nociceptive synaptic terminals in the dorsal horn of the spinal cord

IP_3R2: type 2 inositol triphosphate receptor

Constitutive activity: basal receptor activity in the absence of agonist

Given the limitations to the available tools, we have put considerable effort toward the development of novel approaches to stimulate or remove astrocyte signaling cascades. To stimulate Gq GPCR signaling cascades only in astrocytes, we developed a transgenic line of mice in which a Gq GPCR (MrgA1R) not endogenously expressed in the brain is targeted specifically to astrocytes (44) (**Figure 3**). Although this approach still leads to widespread astrocyte Ca^{2+} elevations, the confound of direct neuronal stimulation (and potential stimulation of many other cell types) by the applied agonist is greatly reduced or eliminated. To study the effects of removing astrocyte spontaneous and agonist-evoked Ca^{2+} elevations, we have utilized IP_3 receptor type 2 (IP_3R2) knockout (KO) mice (see below). These transgenic techniques offer certain improvements over the traditional approaches. However, they also have the potential to introduce new problems. For example, owing to the strength of promoters used in transgenic constructs, the transgenic receptors are almost certainly overexpressed. This has led to problems with particular receptors that show a high level of constitutive activity (45). However, astrocytic expression of the MrgA1 Gq GPCR does not lead to impairments at the behavioral or cellular level, nor to changes in basal Ca^{2+} activity (44), suggesting that, in the absence of agonist, MrgA1R activity is low.

EVIDENCE FOR ASTROCYTE GLUTAMATE VESICLES AND Ca^{2+}-DEPENDENT EXOCYTOSIS

Although astrocytes have been shown to release transmitters by several Ca^{2+}-independent mechanisms, including reversal of glutamate uptake (46), connexin hemichannels (47), pore-forming P2×7 receptors (48, 49), and swelling-induced activation of volume-regulated anion channels

Figure 3

The Gq-coupled receptor MrgA1 is expressed exclusively by astrocytes in the hippocampus. (*a*) Crossing human glial fibrillary acidic protein-tet transactivator (hGFAP-tTA) mice to tet operon (tetO)-MrgA1 mice resulted in the expression of MrgA1 exclusively in astrocytes in the absence of doxycycline. As green fluorescent protein (GFP) is fused to the MrgA1 receptor construct, GFP staining indicates MrgA1 receptor expression. (*b*) In the hippocampus, 80–90% of astrocytes expressed the MrgA1 receptor whereas neurons did not. The left panel shows astrocytes immunostained for GFAP (*red*) and GFP (*green*) to show MrgA1 expression. Astrocytes coexpressing GFAP and the MrgA1 receptor appear yellow. A few $GFAP^+$ astrocytes do not express the MrgA1 receptor (*arrows*). The right panel shows GFP labeling in astrocytes (*green*) together with neuronal nuclei (NeuN labeling in stratum pyramidale of CA1) (*red*). Neurons do not express the MrgA1 receptor. A few astrocytes in the field do not express MrgA1 (*arrows*). (*c*) Nerve-glial antigen 2 ($NG2^+$) glia do not express the MrgA1 receptor. Arrows point to regions of minimal GFP staining (*upper left panel*) that correspond to specific $NG2^+$ antibody labeling (*lower left panel*). In the merged image (*right panel*), GFP and $NG2^+$ staining do not overlap. (*d*) A higher magnification image showing overlapping expression of GFAP and GFP. Arrowheads point to cells expressing both GFAP and GFP, demonstrating that astrocytes express the MrgA1 receptor. Scale bars, 20 μM. Reprinted with permission from Elsevier Limited, copyright 2007 (44).

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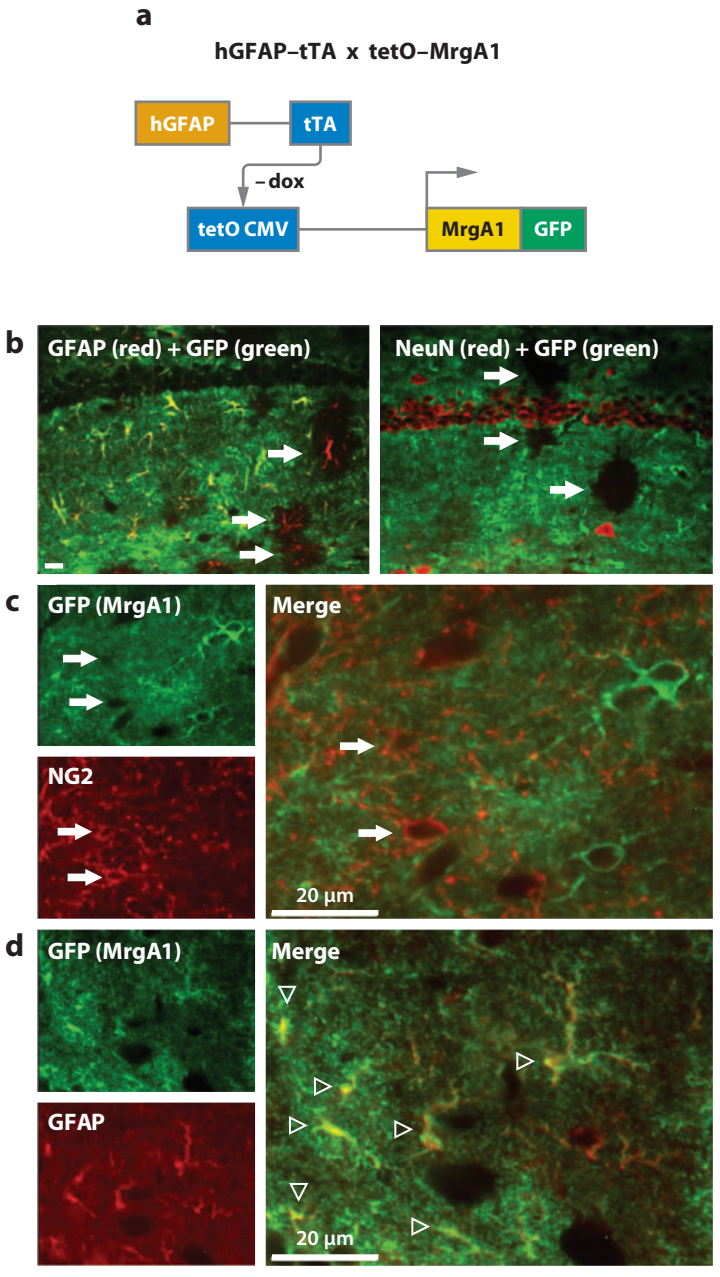
(50–52), vesicular release has received the most attention because of its Ca^{2+}-dependency and potential to occur under physiological conditions. Therefore, in our discussion of whether astrocytes modulate various activities in the brain via Ca^{2+}-dependent release of gliotransmitter, it is important to review available evidence concerning the existence and composition of astrocytic vesicular organelles. This section focuses on astrocytic vesicles containing glutamate, due to the considerable attention vesicular glutamate release by astrocytes has received in the literature. The general approach to studying Ca^{2+}-dependent exocytosis machinery in astrocytes



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Kiss-and-run: vesicular fusion in which only partial fusion of the vesicle with the target membrane occurs, resulting in release of only a fraction of the vesicular content

v-SNARE: SNAREs localized to the synaptic vesicle membrane

has been to assay for proteins known to play a role in this process in neurons. It is worthwhile to point out that study of vesicular release in neurons is intense and ongoing. On the basis of the emerging essential roles of other proteins for exocytosis (53, 54), there is still considerable debate surrounding the model that the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) constitute the minimal membrane fusion machineries.

For an astrocyte to release vesicular organelles containing glutamate in a Ca^{2+}-dependent manner, it is generally thought that four major components are required: (*a*) the vesicles themselves, (*b*) the essential SNARE proteins, (*c*) a vesicular Ca^{2+} sensor (the synaptotagmins), and (*d*) a means for loading glutamate into the vesicular lumen [the vesicular glutamate transporters (VGLUTs)]. Evidence has accumulated, predominantly from work performed on cultured astroglia (for an extensive review see Reference 55), that astrocytes possess at least some version(s) of all four of these essential components. However, several differences exist between astrocytes and neurons that either suggest radically different characteristics, and therefore functions, of vesicular glutamate release by astrocytes or provide uncertainty as to whether Ca^{2+}-dependent exocytosis of glutamatergic vesicles occurs at all in astrocytes.

Astrocytes do not possess active zones, areas of electron-dense material comprised of various proteins that are crucial for normal neurotransmitter release [e.g., Munc13s and RIMs, the Rab3 interacting molecules (53)]. The active zone is where neuronal synaptic vesicles cluster, dock, and become primed for Ca^{2+} responsiveness (54). There is as yet no evidence for Munc13, Munc18, or Rab3 in astrocytes in situ. Thus it is unclear what specific proteins might be involved in directing astrocytic vesicles to specific sites in processes adjacent to synapses and priming them for release. Astrocytic synaptic-like microvesicles (SLMVs) observed in situ are seven- to ninefold less dense in astrocyte processes compared with neuronal synaptic vesicles (SVs) in presynaptic boutons (9, 56). These observations indicate a much more diffuse distribution of astrocyte vesicles, likely resulting in very different spatial and temporal characteristics of release. In neurons, even a locally high density of primed readily releasable SVs at the active zone has a low probability of release (10–20%) following action-potential mediated depolarization and fast Ca^{2+} entry via voltage-gated Ca^{2+} channels. Astrocyte Ca^{2+} elevations, driven by Ca^{2+} release from IP_3-sensitive internal stores, are much slower and longer lasting (in seconds as opposed to microseconds) by comparison. This would be more akin to residual Ca^{2+} in neurons, which plays a role in asynchronous or delayed exocytosis.

The putative astrocyte vesicular Ca^{2+} sensor is synaptotagmin IV (Syt IV), which in one study has been observed in approximately 22% of S-100β^+ processes in situ (57). Syt IV may favor a kiss-and-run mechanism of release over full vesicular fusion (57). Kiss-and-run results in only partial release of the vesicular content, placing a limitation on the concentration of glutamate that might result from individual fusion events of astrocytic SLMVs. Thus, Syt IV expression and functionality suggest more of a modulatory or perhaps housekeeping role of astrocyte vesicular glutamate release. This hypothesis is supported by the work of Ferguson et al. (58), who demonstrated that complete knockout of Syt IV does not affect the basal properties of neurotransmission or release probability in the hippocampus. This led the authors to conclude that Syt IV is nonessential, but plays a modulatory role in hippocampal neurotransmission, an observation that is in agreement with the general well-being of the Syt IV–deficient mice (58). Overall, the slow Ca^{2+} kinetics, low density of SLMVs, lack of active zones, and kiss-and-run release all favor a diffuse, asynchronous, low probability, and low concentration of astrocyte vesicular glutamate release relative to neuronal vesicular release (see **Table 1**).

However, even a modified version of Ca^{2+}-dependent vesicular glutamate release by astrocytes is questionable given considerable discrepancy in the literature with regard to the expression of essential vesicular release machinery by astrocytes, the ability of Syt IV to sense Ca^{2+}, and the expression of VGLUTs. The essential SNARE components in neurons are the v-SNARE

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Table 1 Comparison of neuronal versus astrocytic glutamatergic vesicles and release machinery in situ

	Neurons	Astrocytes
Presynaptic boutons	Yes	No
Presynaptic active zone	Yes	No
Vesicle density	~200 per bouton (~1 μm^3) in multiple pools; 5–10 docked at active zone[a]	Seven- to ninefold less dense in processes; diffuse[b]
Vesicle docking and priming	Munc 13, Munc 18, Rab3 interacting molecule (RIM)	Unknown
Ca^{2+} source for release	Voltage-gated Ca^{2+} channels (VGCCs)[c]	IP_3-sensitive internal Ca^{2+} stores
Ca^{2+} sensor	Synaptotagmin I (Syt I)	Syt IV[d,e]
SNARE minimal secretory apparatus		
v-SNARE	Synaptobrevin (VAMP) 1,2	VAMP2[d]
t-SNARES	Syntaxin I, SNAP-25	Unknown, SNAP-23
Glutamate transporter	VGLUTs 1 and 2, VGLUT3 in nonglutamatergic neurons	VGLUT3 (see text)
Estimated vesicular glutamate concentration	60 mM	20 mM[f]
Release probability (Pr)	~20% when action potential invades presynaptic terminal	Unknown
Type of release	Full fusion or kiss-and-run	May favor kiss-and-run (releases only fraction of vesicular content)
Glutamate concentration following release	1.1 mM (in cleft)[g]	1–100 μM near astroglial processes in vitro[h]
Role in brain signaling	Fast synaptic transmission, synaptic plasticity	Still being explored

[a]Reference 110.
[b]References 9 and 56.
[c]Miniature release may be store Ca^{2+} regulated (111).
[d]Expression in astrocytes is controversial, see text.
[e]Syt IV may not act as a Ca^{2+} sensor in mammals (63).
[f]Reference 55.
[g]Reference 112.
[h]Reference 113.

synaptobrevin (VAMP) and the t-SNAREs, syntaxin and SNAP-25 (54). There is no evidence for expression of syntaxin and SNAP-25 other than in cultured astroglia, which almost certainly express a subset of vesicular molecules not expressed by astrocytes in situ (59). Wilhelm et al. report expression of VAMP2 in astrocytes in situ; however, dominant negative expression of VAMP2 in astrocytes (8) has surprisingly not been linked to alterations in glutamate release by astrocytes. Further complicating the issue is that in a recent study cataloging expression levels of more than 20,000 genes in astrocytes, oligodendrocytes, and neurons, no evidence was found for expression of VAMP2 or Syt IV in purified astrocyte preparations (60; B.A. Barres, personal communication). Instead of SNAP-25, astrocytes may express at low levels SNAP-23, a SNAP-25 family member of which little is known. Some evidence suggests that SNAP-23 functions in docking/fusion of secretory granules in endocrine cells at low Ca^{2+} (61), and in GLUT4 trafficking in adipocytes (62). Expression of SNAP-23 may therefore provide many different cell types with a mechanism for constitutive or regulated secretion at basal levels of intracellular Ca^{2+}.

Interestingly, it is not clear that Syt IV acts as a Ca^{2+} sensor in mammals. Syt IV, like many of the synaptotagmins other than Syts I and II, has a mutation in its C_2A Ca^{2+}-binding domain

VAMP2: v-SNARE vesicle-associated membrane protein, also called synaptobrevin

t-SNARE: SNAREs localized to the target membrane

SNAP-25: t-SNARE synaptosome associated protein of 25 kDa

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that renders this region unable to bind Ca^{2+}. This finding led to the assumption that Syt IV can bind Ca^{2+} only via its C_2B domain. Dai et al. (63) have provided data to suggest, however, that changes in the orientation of critical Ca^{2+}-binding domains and perhaps also their flexibility make the mammalian Syt IV C_2B domain unable to form full Ca^{2+}-binding sites. This would render Syt IV completely unable to bind Ca^{2+}. These findings about Syt IV raise questions about the Ca^{2+}-dependence of vesicular glutamate release by astrocytes. Perhaps Syt IV and SNAP-23 are involved in constitutive, Ca^{2+}-independent gliotransmitter release by astrocytes that contributes to ambient levels of extracellular transmitter such as glutamate, D-serine, and adenosine (64, 65).

SNAP-23: SNAP-25 family member found in nonneuronal cells that might function at basal Ca^{2+} levels

Cholecystokinin (CCK): a neuropeptide found in a subset of GABA interneurons

There is also considerable discrepancy in the literature regarding expression of the VGLUTs, transporters that are necessary for loading vesicles with glutamate. Zhang et al. (66) and Bezzi et al. (56) report expression of VGLUTs 1 and 2 by astrocytes in situ, whereas other studies find that astrocytes express only VGLUT3 but not VGLUTs 1 or 2 (67–69), or do not express any of the VGLUTs (70). These discrepancies may be due in part to a lack of specificity of the probes and antisera used in the different studies (70). Nevertheless, the majority of data indicate that VGLUTs 1 and 2 are expressed exclusively in asymmetrical synaptic terminals of excitatory neurons and not in glia (60, 67, 68, 70, 71). Although VGLUT3 has been observed in an undefined subset of astrocytes, primarily in the white matter (68), there is a general consensus that VGLUT3 labeling is much stronger in discrete neuronal populations, including serotonergic and cholinergic long projection neurons from the dorsal and median raphe nuclei and basal forebrain, respectively, and in local projection GABA interneurons, including cholecystokinin-(CCK) positive basket cells in the hippocampus (67, 68, 70–73). It is interesting to speculate about the physiological implications of the potential release of glutamate by inhibitory GABA interneurons. Recently, Seal and coworkers (74) have uncovered important functional roles of VGLUT3 glutamatergic vesicles. VGLUT3 knockout results in deafness in mice due to loss of glutamate release by inner hair cells of the organ of Corti, which normally express only VGLUT3 glutamatergic vesicles. Furthermore, these mice exhibit seizure-like EEG activity with remarkably little change in ongoing motor behavior, an effect the authors speculated may be due to loss of glutamate release by GABA interneurons and/or serotonergic long projection neurons in the cortex (74). Although not discussed specifically, these findings leave open the possibility that loss of VGLUT3 vesicular organelles in a subset of astrocytes might also contribute to the increased seizure activity. It would be interesting to see what effects, if any, might result from an astrocyte-specific inducible knockout of VGLUT3. VGLUT3 in astrocytic vesicles might also (*a*) help load secretory vesicles with a different transmitter, such as the synergism between monoamine and ATP transport (68); (*b*) serve to create an intracellular storage organelle to buffer cytoplasmic glutamate or other toxins for housekeeping purposes (67, 68); or (*c*) fulfill a different role other than loading vesicles with glutamate, as VGLUTs were originally discovered as members of the Na^+-dependent phosphate transporter family (67, 70).

Overall, on the basis of available evidence, it is very difficult to conclude that astrocytes possess the right combination of machinery required for Ca^{2+}-dependent release of glutamatergic vesicles. Although some astrocytes, mainly in white matter, appear to express VGLUT3, it is not clear that the minimal SNARE apparatus necessary for vesicular release is complete or functional. Several reports indicate that astrocytes do not express syntaxin; SNAP-25; Syts I, II, or IV; or VAMPs 1 or 2. The likelihood of an essential role for SNAP-23 and Syt IV in Ca^{2+}-dependent glutamate release by astrocytes is diminished by the possibility that these proteins function in a Ca^{2+}-independent manner. The low density of astrocytic SLMVs compared with neuronal SVs, the lack of active zones, and a possible association with Syt IV and the VGLUT3 transporter point to a modulatory or housekeeping (although still potentially important) role of astrocyte vesicular glutamate release. Astrocytic glutamatergic vesicular organelles may normally serve a storage purpose and/or be constitutively released asynchronously at low frequency, low density, and low probability to

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help maintain extracellular transmitter tone. Use of our pharmacological approaches to stimulate astrocyte Ca^{2+} (outlined above) may evoke a surge of vesicular fusion that is nonphysiological.

DO ASTROCYTES MODULATE NEURONAL ACTIVITY BY Ca^{2+}-DEPENDENT RELEASE OF GLUTAMATE?

Slow inward current (SIC): Nonsynaptic NMDA receptor-mediated current characterized by large amplitudes and slow kinetics

It is unequivocal that astrocytes play a significant role in the modulation of excitatory synaptic activity in many brain regions. Fine astrocyte processes ensheath pre- and postsynaptic elements (75), and they help prevent neuronal hyperexcitability by taking up extrasynaptic glutamate and potassium (K^+) via glutamate transporters (76) and inwardly rectifying K^+ channels (77), respectively. Astrocytes are also essential for neuronal metabolic activity, possessing specific enzymes for synthesis of tricarboxylic acid (TCA) cycle intermediates on which neurons rely for energy production (78). Fluoroacetate inhibition of astrocytic metabolism of citrate to isocitrate, and thus the formation of α-ketoglutarate from oxaloacetate, severely suppresses glutamatergic transmission by impairing neuronal synthesis of glutamate from glucose (78). These astrocyte functions are critical for maintaining healthy brain activity.

Astrocytes are also reported to play a significant role in modulating neuronal excitatory synaptic activity by Ca^{2+}-dependent release of the gliotransmitters glutamate and ATP. Although this section focuses on glutamate release because this has been an area of active research in our lab for many years, much of the discussion that follows might be applicable to any gliotransmission that is reported to be Ca^{2+}-dependent, including astrocytic release of ATP (79) and D-serine (80).

Two predominant effects in neurons in situ have been attributed to astrocyte Ca^{2+}-dependent release of glutamate using the standard approaches: an increased probability of neurotransmitter release from neuronal presynaptic terminals (7, 9, 13, 81, 82) and postsynaptic neuronal NMDAR-mediated slow inward currents (SICs) (3–6, 31). Because these studies involve either direct uncaging of Ca^{2+} or IP_3 in astrocytes (3, 7, 13), or loss of the neuronal modulation by chelating astrocyte Ca^{2+} or by emptying astrocyte Ca^{2+} stores (81–83), it has been concluded that astrocyte Ca^{2+} is not only necessary but is also sufficient by itself for the astrocytic release of glutamate. This is the same conclusion derived from a large body of earlier work performed on cultured astroglia (for a review see Reference 84). However, although we observed a Ca^{2+}-dependent astrocytic modulation of presynaptic release by uncaging IP_3, for years our group has been unable to find evidence for astrocyte-driven neuronal SICs in situ using uncaging (13) and agonist-based approaches (44; T. Fiacco & K. McCarthy, unpublished data). As outlined above, a major problem limiting the effectiveness of agonist-based approaches to stimulate astrocyte Ca^{2+} is that the vast majority of agonists used will directly stimulate endogenous receptors on neurons and other cells in the tissue. To enable selective stimulation of astrocyte Ca^{2+}, we expressed a Gq GPCR in astrocytes (the MrgA1R) that is not endogenously expressed in the brain, and the ligand of which does not activate endogenous brain receptors. Stimulation of the astrocyte-specific MrgA1 Gq GPCRs produces widespread, robust Ca^{2+} increases in 90% of hippocampal astrocytes without affecting presynaptic release probability or producing SICs in CA1 pyramidal neurons (44). Together with data using agonists to endogenous astrocytic Gq GPCRs [e.g., endothelins to endogenous endothelin receptors (EtRs)] (**Figure 4**), these findings have provided us with strong evidence that astrocytes are not releasing glutamate in a Ca^{2+}-dependent manner when Gq GPCRs are stimulated.

In lieu of our findings, it was recently suggested that not all Ca^{2+} increases are equivalent in their ability to evoke release of gliotransmitters from astrocytes (85). This is quite a departure from the earlier view that Ca^{2+} alone is sufficient to trigger gliotransmitter release. Calcium appears to be sufficient when it is evoked out of context from other signaling molecules using uncaging approaches or strong electrical depolarization of astrocytes. This is supported by our

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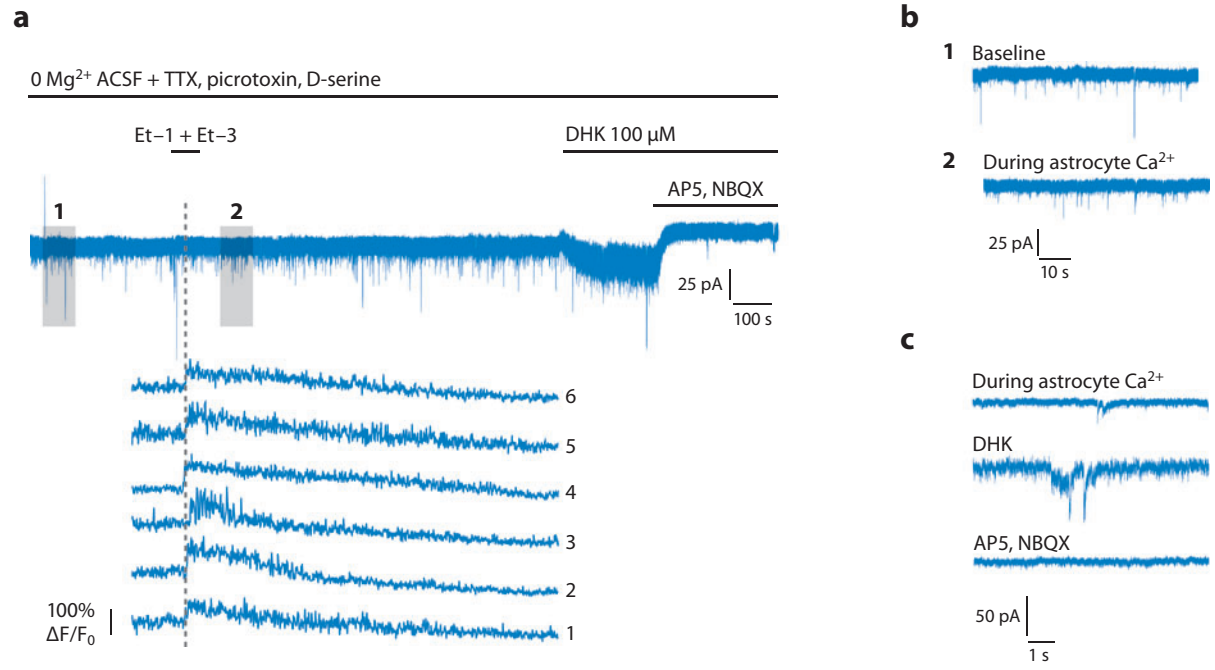

Figure 4

No effect of endothelin receptor-stimulated astrocyte Ca^{2+} elevations on neuronal activity in wild-type mice. (*a*) During neuronal recording of N-methyl-D-aspartate/alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid miniature excitatory postsynaptic currents (NMDA/AMPA mEPSCs) (*upper trace*), astrocyte Ca^{2+} elevations were evoked by application of endothelin-1 (Et-1) and endothelin-3 (Et-3) (*lower traces*, *1–6*). There were no changes observed in the level of spontaneous ionotropic glutamate receptor (iGluR) synaptic activity or in the level of synaptic noise during the astrocyte Ca^{2+} elevations compared with baseline neuronal recording. However, application of the astrocytic glutamate 1 (GLT-1) transporter inhibitor dihydrokainate (DHK) (100 μM) produced an increase in inward current as well as an increase in the level of synaptic noise. Application of DL-2-amino-5-phosphonopentanoate (DL-AP5) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) blocked the increase in iGluR activation by DHK as well as a tonic inward current that was present prior to DHK application. (*b*) Portions of the neuronal recording during baseline (1) versus during astrocyte Et-evoked Ca^{2+} elevations (2) are shown in an expanded timescale for easier comparison. There was no effect of the astrocyte Ca^{2+} elevations on the neuronal recording. (*c*) Comparison of the level of synaptic noise during astrocyte Ca^{2+} elevations (*upper trace*), during DHK administration (*middle trace*) and in the presence of DL-AP5 + NBQX (*lower trace*) shown in expanded timescale reveals no effect of astrocyte Ca^{2+} elevations on the level of iGluR mediated synaptic noise. These data were replicated in five recordings from five hippocampal slices. Reprinted with permission from Elsevier Limited, copyright 2007 (44).

finding that uncaging IP_3 in single MrgA1R$^+$ astrocytes reproduced an increased frequency of neuronal spontaneous excitatory postsynaptic currents (sEPSCs) as seen previously in wild-type (WT) mice (13, 44). The interpretation is that bypassing signaling cascades by direct stimulation of IP_3 or Ca^{2+} produces pharmacological or unchecked Ca^{2+} increases that lead to gliotransmitter release. Signaling molecules activated by Gq GPCR stimulation might provide important checks for regulating Ca^{2+}-dependent release by astrocytes. For example, the $\beta\gamma$ subunits of G proteins have been shown to inhibit neurotransmitter release from presynaptic terminals downstream of voltage-gated Ca^{2+} channels (86). Furthermore, Andric et al. (87) showed that stimulation of EtRs inhibits prolactin release from pituitary lactotrophs in vitro by activation of a G_z signaling pathway. It is important to note, however, that this inhibition occurred only after 5–10 min of strong enhancement of release, presumably owing to activation of Gq signaling pathways (**Figure 5**). These findings suggest that the precise signaling cascade associated with each Gq GPCR subtype

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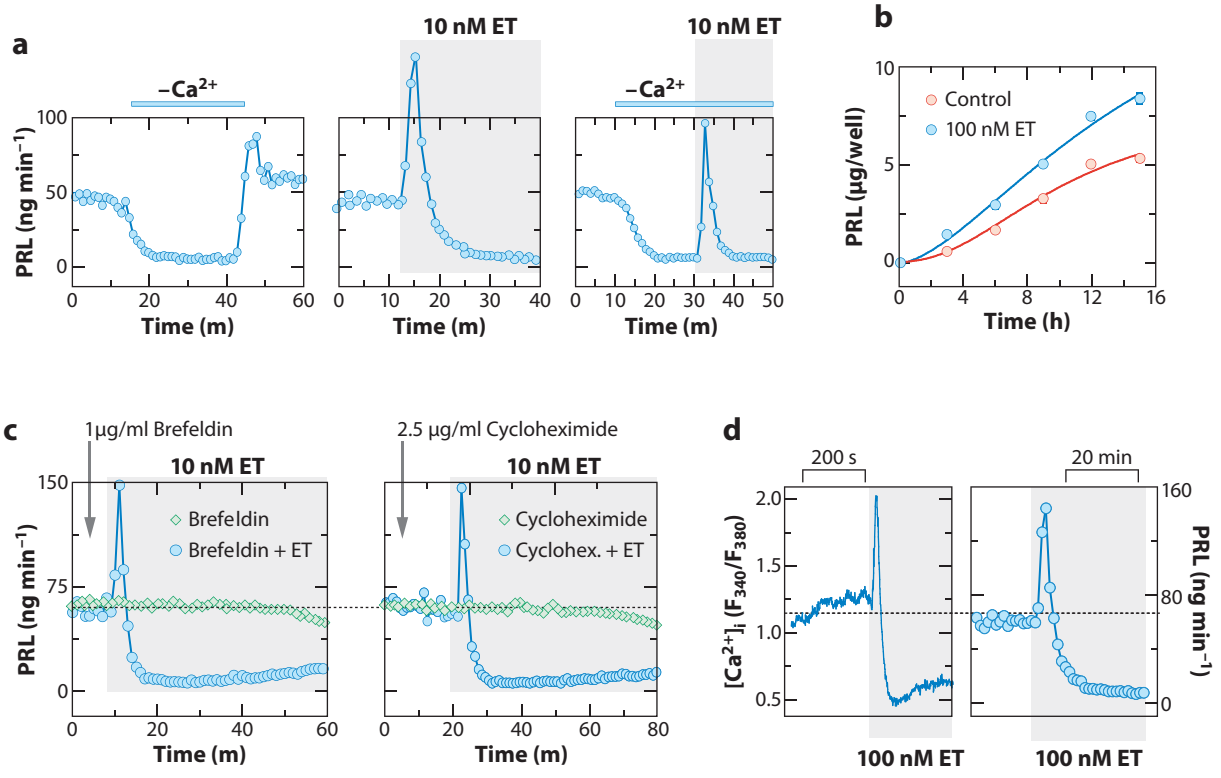

Figure 5

Characterization of endothelin-(Et) induced Ca^{2+} signaling and secretion in pituitary lactotrophs. (*a*) (*Left*) Extracellular calcium dependence of basal prolactin release in perfused pituitary cells; representative trace from eight experiments. (*Middle*) Time course of Et-induced prolactin (PRL) release in pituitary cells perfused with Ca^{2+}-containing medium, representative trace from 36 experiments. (*Right*) Time course of Et-induced PRL release in pituitary cells perfused with Ca^{2+}-deficient medium, representative trace from three experiments. (*b*) Time course of PRL release in static pituitary cells in the presence and absence of Et-1 (mean values with $n = 6$; S.E. are within *circles*). (*c*) The lack of effect of inhibitors of protein synthesis, brefeldin and cycloheximide (*Cyclohex.*), on Et-induced PRL release. (*d*) Bidirectional effects of Et-1 on calcium signaling (*left*) and PRL release (*right*). The averaged Ca^{2+} profile was obtained from 15 single lactotrophs, and the secretory profile was generated from five perfusion experiments. In this and the following figures, gray areas indicate the duration of Et-1 application. Secretory studies were done in unpurified cells, and Ca^{2+} measurements were done in single identified lactotrophs. Reprinted with permission from The American Society for Biochemistry and Molecular Biology, copyright 2005 (87).

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may be important to whether gliotransmitter release occurs. Thus, it is possible that only specific astrocytic Gq GPCRs are coupled to gliotransmitter release, painting a much more complex picture of the mechanisms regulating gliotransmitter release by astrocytes. One of these special astrocytic Gq GPCRs might be metabotropic GluR5 (mGluR5) (85). mGluR5 activation, which has been linked to astrocytic Ca^{2+}-dependent modulation of neuronal activity (although with the caveat of stimulating many direct effects in neurons as discussed above), sometimes produces an oscillatory (rather than a plateau) Ca^{2+} response in astrocytes. This may be due to an oscillatory translocation and activation of protein kinase C (88). The frequency as well as the amplitude of changes in Ca^{2+} concentration following group 1 mGluR stimulation have been shown to be important in determining the pattern of transcription factor activation and gene expression (88). The compound mGluR Ca^{2+} response in astrocytes might also code for gliotransmitter release.

A very specific role of mGluR5 in astrocytes to the release of gliotransmitters could perhaps be explored using an astrocyte-specific knockout of mGluR5. In conclusion, we may need to shift our thinking away from Ca^{2+} as an independent signaling molecule to Ca^{2+} as one player activated within a fabric of other signaling molecules that together play an integrated role in mediating a cellular response.

Although differential Ca^{2+} codes provide a potential explanation for the variable effects of Gq GPCR agonists on gliotransmitter release, we have recently obtained evidence that complete removal of all spontaneous and Gq GPCR agonist-evoked IP_3R-mediated astrocyte Ca^{2+} elevations has no affect on basal CA1 pyramidal neuron miniature and spontaneous excitatory synaptic activity (44, 89). Removal of IP_3R-mediated Ca^{2+} increases in astrocytes was accomplished by knockout of the type 2 IP_3R. IP_3R2 appears to be the only IP_3R isoform expressed by hippocampal astrocytes, whereas neurons predominantly express IP_3R1 (90, 91). Removal of IP_3R2 completely abolishes astrocyte agonist-evoked and spontaneous Ca^{2+} elevations, but leaves neuronal IP_3R-mediated Ca^{2+} elevations intact (**Figure 6**). A detailed behavioral assessment of these mice is ongoing, but the mice appear healthy, breed well, live normal lifespans, and exhibit no overt abnormalities in brain cytoarchitecture or behavior. Furthermore, basal miniature and spontaneous AMPA and NMDA receptor currents are completely unchanged in the IP_3R2 KO mice compared with littermate controls. The overall well-being of these mice calls into question how essential astrocyte Ca^{2+} elevations may be for driving neuronal synaptic transmission, modulating LTP, and regulating neurite outgrowth during cortical development. If astrocytes are significantly involved in the regulation of these processes, one might expect that complete removal of astrocyte Ca^{2+} elevations would lead to profound physiological and behavioral abnormalities. In summary, it is not settled that astrocytes release glutamate in a Ca^{2+}-dependent manner to modulate numerous essential brain activities, including excitatory neuronal synaptic transmission.

CELL SWELLING AS A POTENTIAL MECHANISM FOR ASTROCYTE GLUTAMATE RELEASE

Cell swelling and release of glutamate via volume-sensitive organic anion channels (VSOACs) should be considered a potential mechanism behind some phenomena attributed to

Figure 6

Knockout (KO) of type 2 inositol triphosphate receptor (IP_3R2) affects astrocyte but not neuronal G protein–coupled receptor (GPCR)-mediated Ca^{2+} increases. (*a*) Representative Ca^{2+} traces from astrocytes of Calcium Green 1-AM–loaded hippocampal slices. Regions of interest were placed over the cell bodies of bulk-loaded hippocampal astrocytes to measure Ca^{2+} increases in response to agonist application (*upper panels*). Application of ATP (100 μM) or a G_q-linked GPCR agonist cocktail [Ct: 10 μM dihydroxyphenylglycine (DHPG), 10 μM histamine and 10 μM carbachol] elicited Ca^{2+} responses in astrocytes from littermate control but not IP_3R2 KO hippocampal slices. The arrows indicate the astrocyte Ca^{2+} traces shown in the lower panels. Thapsigargin (Tg, 2 μM) was used as a control and increased Ca^{2+} in astrocytes of both littermate control and IP_3R2 knockouts. Data presented as fold increases over baseline. (*b*) Percentage of astrocytes responding to application of ATP or the G_q-linked GPCR agonist cocktail from all experiments. (*c*) Representative Ca^{2+} traces from CA1 pyramidal neurons patch clamped with internal solution containing Fluo-4 Ca^{2+} indicator in response to application of a G_q-linked GPCR cocktail (50 μM DHPG, 10 μM histamine, 10 μM carbachol) in the presence of 1 μM tetrodotoxin (TTX) to block action potentials. (*d*) Amplitude and duration of IP_3R-mediated Ca^{2+} responses in CA1 pyramidal neurons (Control $n = 8$; IP_3R2 KO $n = 6$). There were no significant differences for amplitude (*left panel*; $p = 0.39$) or duration (*right panel*; $p = 0.08$). Error bars indicate SEM. Reprinted with permission from The Society for Neuroscience, copyright 2008 (89).

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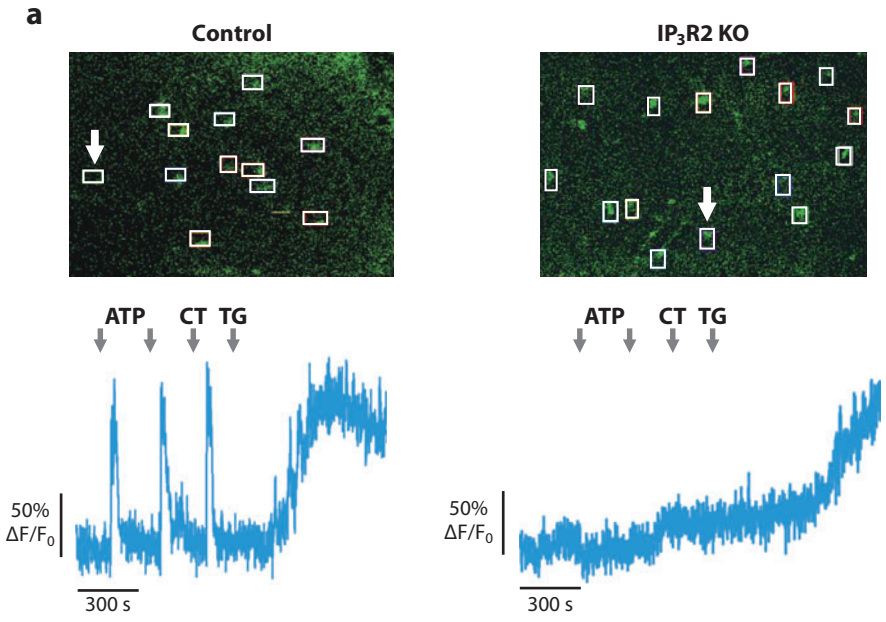
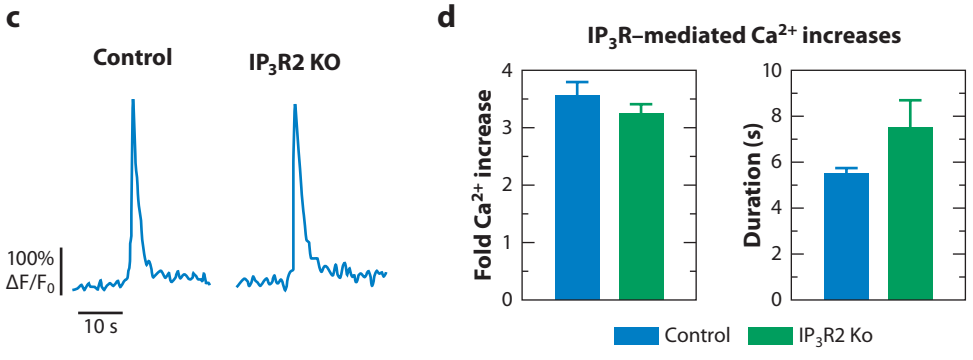
Ca^{2+}-dependent vesicular astrocytic glutamate release. There is little debate that cell swelling occurs during the onset of several neuropathological episodes, including ischemia (stroke), migraine, hyponatraemia, brain trauma, and epilepsy (50, 92–96). Cell swelling occurs as a result of Na^+, Cl^-, and Ca^{2+}, with osmotically obligate water entering neurons and K^+, Cl^-, and water entering astrocytes (92). Swollen cells attempt to regain their normal volume by releasing osmolytes,



b

Treatment	Control	IP_3R2 Ko
ATP	80.0% (32/40 cells)	0% (0/38 cells)
Cocktail (Ct)	65.0% (93/144 cells)	0% (0/104 cells)
Thapsigargin (Tg)	45.0% (18/40 cells)	45.0% (17/38 cells)



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including taurine, glutamate, aspartate, and other amino acids (94). This response is called the regulatory volume decrease (RVD) and is mediated by VSOACs. Because pronounced cell swelling is predominantly seen in astrocytes during brain pathology, it is perhaps not surprising that moderate to severe (20–30%) reduction in solution osmolarity is associated with a 10–20-fold increase in excitatory amino acid (EAA) release from cultured astroglia via the volume-regulated anion channel (VRAC) (50). Thus, astrocytes may play an important role in the exacerbation of excitotoxicity during neurological disorders by releasing glutamate via VSOACs during cell swelling.

Ca^{2+} code: a precise pattern of Ca^{2+} increase or oscillation

VSOAC: volume-sensitive organic anion channel

VRAC: volume-regulated anion channel

Hyponatraemia: a condition defined by a low serum sodium concentration (<130 mM) brought about by excessive water intake

What about the effects of mild astrocyte swelling? Remarkably, Harold Kimelberg's group has shown that a 5% reduction in solution osmolarity leads to a 20–45% increase in EAA release from cultured astroglia over the basal levels (97). Because mild astrocyte swelling occurs alongside nonpathological neuronal excitation (98), likely due to the uptake of released K^+ and glutamate from neurons (99, 100), astrocytes have the potential to release glutamate via activated VSOACs in physiological conditions simply as a byproduct of the regulation of changes in their cell volume. Even in isosmotic conditions, when cells are presumed to have a normal volume, some VSOACs may be active (99). This appears to be the case in the supraoptic nucleus, a specialized nucleus of the hypothalamus involved in secretion of oxytocin and vasopressin. Here, astrocytic VRACs are tonically active in nonswollen cells and mediate taurine release. Via glycine receptor activation, VRACs regulate the secretion of vasopressin by magnocellular neurons (101, 102). Thus, astrocytes have the ability to release glutamate in a nonvesicular, Ca^{2+}-independent manner in physiological conditions during nonswelling or mild swelling conditions.

In addition to the potential for VSOACs to be active under basal conditions, data in vitro and in situ suggest that certain neuromodulators, such as ATP (97, 99, 103) and thrombin (104), potently enhance EAA release from astrocytic VRACs in nonswollen cells. It has also been reported recently that group 1 mGluRs exacerbate astrocyte swelling by acting on the aquaporin 4 channel (AQP4) (105). Although swelling-induced activation and release of EAAs from VSOACs is a Ca^{2+}-independent process (44, 95, 100, 106), ATP and thrombin potentiation of glutamate release from astrocytic VSOACs and group 1 mGluR modulation of AQP4 appear to be Ca^{2+}-dependent. This suggests that astrocyte Ca^{2+} elevations may modulate swelling-induced release of glutamate. Potentiation of VRACs by ATP and group 1 mGluR exacerbation of swelling are especially noteworthy findings, given that these particular agonists have been used extensively to stimulate astrocyte Ca^{2+}-driven neuronal SICs (3, 4, 6, 49, 107). Release of glutamate through VSOACs may be inadvertently potentiated by using these agonists, resulting in the production of neuronal SICs that have been attributed to astrocyte Ca^{2+}-dependent vesicular release of glutamate. An involvement of VSOACs in glutamate release leading to neuronal SICs is supported by the work of Kozlov et al. (5), who demonstrated in situ that spontaneous, synchronized SICs in olfactory bulb mitral cells are greatly enhanced in hypotonic solution, and almost completely blocked in hypertonic solution. Takano et al. (103) also showed that ATP potentiation of glutamate release from astrocytic VSOACs is eliminated by a 15% increase in bath osmolarity. Not only do these findings support a VSOAC-mediated mechanism for astrocytic release of glutamate but they also strongly argue against vesicular release, which is enhanced in hypertonic solution (108). In fact, hypertonic solution is often used as a tool to increase vesicular fusion (e.g., 109). Overall, the findings suggest that release of glutamate from volume-sensitive channels may account for large neuronal slow inward currents that have been attributed to Ca^{2+}-dependent vesicular release from astrocytes. Because VSOACs are ubiquitously expressed by all cell types, further work is required to determine if astrocytes in situ are a primary source of glutamate from activated VSOACs. The potential for VSOACs to be bona fide regulators of astrocytic glutamate release under physiological conditions in situ and in vivo is also worthy of further exploration.

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SUMMARY AND CONCLUSIONS

The primary goal of this review has been to reassess the nature of astrocyte Ca^{2+} activity in intact tissue under basal conditions and discuss evidence of astrocytic involvement in essential brain processes via Ca^{2+}-dependent release of glutamate. The evoked long-range propagating intercellular Ca^{2+} waves in cultured astroglia that many associate with astrocyte signaling bear little resemblance to intrinsic microdomain astrocyte Ca^{2+} oscillations or synchronous astrocyte Ca^{2+} activity driven by active neuronal networks that occur in situ and in vivo. Astrocytic expression of the essential vesicular release machinery for Ca^{2+}-dependent regulated exocytosis of glutamatergic vesicular organelles is not established, suggesting alternate functions of astrocyte glutamate vesicles that are more modulatory or subtle than driving neuronal synaptic transmission. Some phenomena associated with Ca^{2+}-dependent vesicular release of glutamate by astrocytes might be better explained by Ca^{2+}-independent, nonvesicular forms of release associated with the regulation of changes in cell volume.

There have been problems associated with the use of traditional approaches to stimulate astrocyte Ca^{2+} during assessment of changes to neuronal activity that either confound interpretation of the results (use of agonists to endogenous Gq GPCRs), or produce questionable effects by bypassing the fabric of signaling molecules normally activated alongside Ca^{2+} in Gq GPCR signaling pathways (use of caged Ca^{2+}/IP_3 or strong electrical depolarization). Stimulation of transgenic and specific endogenous Gq GPCRs in astrocytes is not associated with astrocytic Ca^{2+}-dependent release of glutamate, suggesting that many astrocyte signaling molecules, not just Ca^{2+}, tightly regulate gliotransmitter release and other astrocyte functions. Complete removal of the ability of astrocytes to elevate Ca^{2+} either spontaneously or in response to Gq GPCR agonists does not affect basal neuronal excitatory synaptic activity, brain cytoarchitecture, or whole-animal behavior, questioning how essential astrocyte Ca^{2+} is for the control of many brain processes. Discovering signaling molecules that might regulate Ca^{2+}-dependent gliotransmitter release could shed light on whether astrocytic release of gliotransmitters occurs, and its potential impact on brain physiology.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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