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The Trinity of Ca²⁺ Sources for the Exocytotic Glutamate Release from Astrocytes

Reno C. Reyes and Vladimir Parpura*

¹ Department of Neurobiology, Center for Glial Biology in Medicine, Atomic Force Microscopy & Nanotechnology Laboratories, Civitan International Research Center, Evelyn F. McKnight Brain Institute, University of Alabama, Birmingham, AL 35294

Abstract

Astrocytes can exocytotically release the transmitter glutamate. Increased cytosolic Ca²⁺ concentration is necessary and sufficient in this process. The source of Ca²⁺ for the Ca²⁺-dependent exocytotic release of glutamate from astrocytes predominately comes from endoplasmic reticulum (ER) stores with contributions from both inositol 1,4,5 trisphosphate- and ryanodine/cafeine-sensitive stores. An additional source of Ca²⁺ comes from the extracellular space via store-operated Ca²⁺ entry due to the depletion of ER stores. Here transient receptor potential canonical type 1 containing channels permit entry of Ca²⁺ to the cytosol, which can then be transported by the store-specific Ca²⁺-ATPase to (re)fill ER. Mitochondria can modulate cytosolic Ca²⁺ levels by affecting two aspects of the cytosolic Ca²⁺ kinetics in astrocytes. They play a role in immediate sequestration of Ca²⁺ during the cytosolic Ca²⁺ increase in stimulated astrocytes as a result of Ca²⁺ entry into the cytosol from ER stores and/or extracellular space. As cytosolic Ca²⁺ declines due to activity of pumps, such as the smooth ER Ca²⁺-ATPase, free Ca²⁺ is slowly released by mitochondria into cytosol. Taken together, the trinity of Ca²⁺ sources, ER, extracellular space and mitochondria, can vary concentration of cytosolic Ca²⁺ which in turn can modulate Ca²⁺-dependent vesicular glutamate release from astrocytes. An understanding of how these Ca²⁺ sources contribute to glutamate release in (patho)physiology of astrocytes will provide information on astrocytic functions in health and disease and may also open opportunities for medical intervention.

Keywords

astrocytes; glutamate release; exocytosis; Ca²⁺ ER stores; SOC entry; TRPC1; mitochondria

A developing body of evidence implicates astrocytes as active participants in multi-directional signaling in the central nervous system (CNS) [reviewed in (Haydon and Carmignoto, 2006; Theodosis et al., 2008)]. Astrocytes communicate with neurons (Nedergaard, 1994; Parpura et al., 1994), endothelial cells of the cerebrovasculature (Zonta et al., 2003; Mulligan and MacVicar, 2004), and microglia (Davalos et al., 2005). They appear to be an integral part of neural communication throughout the CNS. For example, in the hippocampus, astrocytes integrate neuronal signals (Perea and Araque, 2005), potentiate transmitter release at single synapses (Perea and Araque, 2007), enhance long-term potentiation (Pascual et al., 2005), and

Correspondence should be addressed to: Vladimir Parpura, Department of Neurobiology, 1719 6th Avenue South, CIRC 429, University of Alabama, Birmingham, AL 35294; phone: (205) 996-7369; fax: (205) 975-6320; e-mail: E-mail: vlad@uab.edu.

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synchronize neuronal activity (Fellin et al., 2004). In the thalamus, astrocytes have been shown to drive neuronal activity as a consequence of astrocytic intrinsic Ca^{2+} oscillations (Parri et al., 2001). In the supraoptic nucleus of the hypothalamus, morphological plasticity of astrocytes (Hatton, 2004) leading to differential synaptic coverage by astrocytic processes can modulate the activity of the output neurons (Pاناتier et al., 2006).

The abundance of astrocytes and their proximity to neurons enables their communication with neurons. Early studies indicated the numerical preponderance of glial cells in the CNS. For example, in layers I and IV of the rat cerebral cortex, glial cells consisting of astrocytes and oligodendrocytes, outnumbered neurons 3 to 1 (Bass et al., 1971). As more sophisticated tools for cell counting emerged, it appears, however, that the glia to neuron ratio in human cortex is ~ 1.65:1, while in rodent this ratio is about 0.3:1 (Nedergaard et al., 2003; Sherwood et al., 2006). Astrocytes occupy distinct domains within the CNS, only at the cells' peripheries do their processes overlap (Bushong et al., 2002; Ogata and Kosaka, 2002). Within their individual domains astrocytes have extensive morphological interactions with neurons. For example, in the cortex of adult mice, one astrocyte may contact 4 to 8 neurons and surround ~300 to 600 neuronal dendrites (Halassa et al., 2007). In the hippocampus, astrocytes are positioned to contact even more synapses. In adult rats, one astrocyte is estimated to contact ~140,000 synapses of CA1 pyramidal cells (Bushong et al., 2002). Classically, the intimate position of astrocytic processes around the neurons and the synapses has been solely attributed to glutamate uptake and regulation of extracellular K^+ by astrocytes. To provide these services, astrocytes possess excitatory amino acid transporters (EAAT's) (Huang and Bergles, 2004; Tzingounis and Wadiche, 2007; Yang and Rothstein, 2009) and K^+ inward rectifying (Kir) channels (Sontheimer et al., 1994; Kofuji and Newman, 2009), respectively. However, it is also at synapses where astrocytes have been shown to have modulatory effects on the activities of presynaptic and postsynaptic neurons by astrocytic ability to release a variety of transmitters using many different mechanisms [reviewed in (Malarkey and Parpura, 2009)]. This led to the concept of a functional tripartite synapse (Araque et al., 1999), a topic that has been recently reviewed elsewhere (Ni et al., 2007; Halassa and Haydon, 2009).

In this review we focus on the Ca^{2+} sources for the exocytotic release of the excitatory transmitter glutamate from astrocytes, which can readily occur under physiological conditions (Parpura and Haydon, 2000). We start with a historical brief on the Ca^{2+} -dependent glutamate release from astrocytes [reviewed in detail in (Montana et al., 2004)]. We then discuss the trinity of Ca^{2+} sources for this release: intracellular stores, extracellular space and mitochondria. An understanding of how these Ca^{2+} sources contribute to the glutamate release will provide information on glial functions in health and disease and may also introduce opportunities for medical intervention.

Ca^{2+} -dependent glutamate release in astrocytes: a historical brief

Astrocytes can couple their inherent intracellular Ca^{2+} excitability to the exocytotic release of transmitters including but not limited to glutamate (Parpura et al., 1994). Throughout this review we interchangeably use Ca^{2+} -dependent and exocytotic attributes of glutamate release from astrocytes. Excitation-secretion coupling in astrocytes involves the molecular machinery for vesicular fusion, including the core members of the soluble N-ethyl maleimide-sensitive fusion protein attachment protein receptor (SNARE) complex, as well as proteins responsible for packaging of glutamate inside vesicles, vacuolar type proton ATPase and vesicular glutamate transporters [reviewed in (Montana et al., 2006; Malarkey and Parpura, 2008)].

Evidence for Ca^{2+} -dependent release of glutamate from astrocytes was originally demonstrated in experiments where the application of the Ca^{2+} ionophore ionomycin stimulated the release of glutamate from astrocytes in the presence of external free Ca^{2+} (2.4 mM). However, this

ionophore failed to cause glutamate release when applied to astrocytes that were equilibrated in solution with low external free Ca^{2+} (24 nM), for 40–60 minutes, thus causing the depletion of internal Ca^{2+} stores and preventing the Ca^{2+} entry from the extracellular space (Parpura et al., 1994). These data indicate that Ca^{2+} is sufficient and necessary to cause glutamate release from astrocytes. Indeed, depleting internal Ca^{2+} stores by application of thapsigargin, a blocker of the sarco-endoplasmic reticulum Ca^{2+} -ATPase (SERCA), or buffering cytoplasmic Ca^{2+} with the membrane permeable Ca^{2+} chelator, 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid acetoxymethyl ester (BAPTA-AM), resulted in reduction of glutamate release (Araque et al., 1998a; Bezzi et al., 1998; Innocenti et al., 2000; Hua et al., 2004; Montana et al., 2004). Furthermore, alternative stimuli that directly increased astrocytic intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$), such as mechanical stimulation (Parpura et al., 1994; Araque et al., 1998a; Araque et al., 1998b; Hua et al., 2004; Montana et al., 2004), photostimulation (Parpura et al., 1994), and photolysis of Ca^{2+} cages (Araque et al., 1998b; Parpura and Haydon, 2000), all caused release of glutamate.

The trinity of Ca^{2+} sources

The source of Ca^{2+} for Ca^{2+} -dependent release of glutamate from astrocytes is tripartite, from (i) internal ER stores, (ii) extracellular space and (iii) mitochondria (Figure 1).

(i) Inositol 1,4,5 trisphosphate (IP_3)- and ryanodine/caffeine-sensitive ER stores

The ER is the major source of intracellular Ca^{2+} , and it appears to be the main determinant of Ca^{2+} excitability in astrocytes (Verkhatsky, 2006; Deitmer et al., 2008). The concentration of free Ca^{2+} inside the ER ($[\text{Ca}^{2+}]_{\text{ER}}$) ranges from 100 to 800 μM (Burdakov et al., 2005) while the basal concentration of this ion in cytosol ($[\text{Ca}^{2+}]_{\text{cyt}}$) is ~ 100 nM. Ca^{2+} can be released from the ER through IP_3 receptors (IP_3R) and Ca^{2+} -induced Ca^{2+} release (CICR) by activation of caffeine/ryanodine sensitive receptors (RyR) (Simpson et al., 1998). These two ER receptor/channel types may gate distinct Ca^{2+} stores (Golovina and Blaustein, 2000), although this notion has not been widely accepted [e.g., (Hua et al., 2004); see below]. The $[\text{Ca}^{2+}]_{\text{ER}}$ can affect the sensitivity of the RyRs to cytosolic Ca^{2+} as well as the activity of the SERCA pumps (Burdakov et al., 2005). Thus, at the level of the ER, there is regulation of Ca^{2+} release and entry.

The initial, but indirect indication that ER stores play role in Ca^{2+} -dependent release of glutamate from astrocytes came from experiments in which the neuroligand bradykinin which caused cytosolic Ca^{2+} elevation in astrocytes also induced glutamate release from these glial cells (Parpura et al., 1994). Bradykinin binds to G-protein coupled plasma membrane receptors which activate the IP_3 pathway, and cause release of Ca^{2+} from the ER (Cholewinski et al., 1988; Stephens et al., 1993). In addition to bradykinin, other ligands including ATP can induce Ca^{2+} release from the ER (Peuchen et al., 1996) through the IP_3 pathway (Kastritsis et al., 1992). Indeed, ATP stimulation of purinergic 2 receptors caused an increase in astrocytic cytosolic Ca^{2+} which coincided with an increase in glutamate release from cultured astrocytes to the extracellular space (Jeremic et al., 2001). This ATP action was greatly reduced when astrocytes were pre-incubated with thapsigargin, implicating the role of ER stores in providing cytosolic Ca^{2+} for exocytotic glutamate release.

Hua et al. (2004) demonstrated that both IP_3 - sensitive and ryanodine/caffeine-sensitive stores play a role in Ca^{2+} -dependent mechanically-induced glutamate release from astrocytes. The Ca^{2+} source for this exocytotic release is predominately from internal stores, as indicated by reduction of mechanically-induced glutamate release in the presence of thapsigargin. To test whether IP_3 - sensitive internal stores mediate Ca^{2+} -dependent glutamate release from astrocytes, these cells were bathed in diphenylboric acid 2-aminoethyl ester (2-APB) solution, a cell-permeant IP_3R antagonist. This agent greatly reduced exocytotic glutamate release. Next,

the role of ryanodine/caffeine-sensitive ER stores was assessed by incubating astrocytes with ryanodine, which at concentrations used (10 μ M) blocked the release of Ca^{2+} from the ryanodine/caffeine-sensitive stores. Ryanodine also attenuated mechanically-induced glutamate release. Furthermore, the sustained presence of caffeine that depleted ryanodine/caffeine stores, also reduced mechanically-induced glutamate release. It should be noted, however, that the functionality of ryanodine receptors in astrocytes is still debated, since the lack of their activity in astrocytes *in situ* had been reported (Beck et al., 2004). Nonetheless, when astrocytes in culture were pre-treated with the combination of 2-APB with ryanodine or caffeine, there was no additive effect on reduction of mechanically-induced glutamate release when compared to treatments with one pharmacological agent only. Since all pharmacological agents used in this study showed cytosolic Ca^{2+} changes that were in good agreement with glutamate release, these data suggested that exocytotic glutamate release requires the co-activation of both IP_3 - and ryanodine/caffeine-sensitive internal Ca^{2+} stores and that these stores are operating jointly (Figure 1).

(ii) Extracellular space

Astrocytes utilize extracellular Ca^{2+} as well as ER Ca^{2+} for exocytotic glutamate release (Figure 1). This is evidenced by the reduction of Ca^{2+} -dependent glutamate release from astrocytes not only in the presence of thapsigargin, but also in the presence of extracellular Cd^{2+} , a blocker of Ca^{2+} entry from the extracellular space (Hua et al., 2004). It should be noted that remaining glutamate release in stimulated astrocytes pre-treated with thapsigargin or Cd^{2+} were ~30% and 55% of control, respectively, indicating that the internal stores are the predominant source of Ca^{2+} for exocytotic release.

Ca^{2+} entry across the astrocytic plasma membrane involves a variety of channels [reviewed in (Verkhatsky et al., 1998); see below]. Ultimately, the (re)filling of ER Ca^{2+} stores requires Ca^{2+} entry from the extracellular space through so called store-operated Ca^{2+} (SOC) channels (Takemura and Putney, 1989; Golovina, 2005). Canonical transient receptor potential (TRPC) channels, which have been implicated in SOC entry, have been found in astrocytes where they play a role in the regulation of Ca^{2+} homeostasis (Pizzo et al., 2001; Grimaldi et al., 2003; Golovina, 2005). TRPC1 functionally contributes to Ca^{2+} -dependent glutamate release from astrocytes. Malarkey et al. (2008) detected the presence of TRPC1 in cultured and freshly-isolated astrocytes, along with two other isoforms with which TRPC1 is known to form heteromultimeric channels, TRPC4 and TRPC5 (Strubing et al., 2001; Hofmann et al., 2002; Strubing et al., 2003). It should be noted that TRPC1 does not commonly form homomeric channels (Strubing et al., 2003). When looking at the function of these proteins, Malarkey et al. (2008) measured the extent of SOC entry in cultured astrocytes. Astrocytic internal Ca^{2+} stores were depleted by applying cyclopiazonic acid (CPA), which blocks SERCA and (re) filling the stores. Concomitantly, the entry of external Ca^{2+} was prevented by bathing the astrocytes in a Ca^{2+} free external saline solution. This depletion of Ca^{2+} from internal stores led to opening of SOC channels, which was evident by the increased cytosolic Ca^{2+} when the bathing solution was changed to external solution containing Ca^{2+} . This increase was pharmacologically confirmed to be due to Ca^{2+} entry through SOC channels using La^{3+} , Gd^{3+} and MRS-1845, compounds known to inhibit SOC channels (Harper et al., 2003; Rychkov and Barritt, 2007).

In experiments using a similar SOC entry assay to the one described above, Golovina (2005) demonstrated significant reduction of SOC entry in cultured astrocytes after an antisense knockdown of the TRPC1 gene. To address whether the acute block of TRPC1 affects SOC entry, Malarkey et al. (2008) used an antibody against TRPC1 that was designed to bind to the pore forming region of TRPC1 and block the functioning of the channel (Wang et al., 1999). This antibody caused a significant decrease in the measured amount of SOC entry in astrocytes.

Such immunological interference with TRPC1 channels also showed that the SOC entry through TRPC1 plays a role in mechanically-induced cytosolic Ca^{2+} elevations and the consequent Ca^{2+} -dependent release of glutamate from astrocytes (Figure 1). The reduction of glutamate release in astrocytes pretreated with antibody was ~ 39% of control, thus at similar level to that seen in astrocytes treated with thapsigargin (Hua et al., 2004).

It is obvious by now that astrocytes are unlike neurons in which the predominant source of Ca^{2+} for exocytotic release at synaptic terminals is extracellular, mediated via Ca^{2+} entry through voltage-gated Ca^{2+} channels (VGCCs). However, VGCCs were found in astrocytes (Latour et al., 2003; Mulligan and MacVicar, 2004). Since the driving force for the for Ca^{2+} entry into astrocytes is similar to that in neurons with the extracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{ext}}$) ~2 mM being four orders greater than $[\text{Ca}^{2+}]_{\text{cyt}}$, the inability to detect the activity of VGCCs in hippocampal astrocytes *in situ* may implicate the low level of expression and/or low plasma membrane density of VGCCs (Carmignoto et al., 1998). Although the role of VGCCs in glutamate release from astrocytes is unclear at present, there is some evidence in support of their involvement in this process. Using freshly prepared slices from ventrobasal thalamus, Parri et al. (2001) showed that astrocytes *in situ* display intrinsic $[\text{Ca}^{2+}]_{\text{cyt}}$ oscillations. These oscillations were not driven by neuronal activity since they could not be blocked by tetrodotoxin. Astrocytes *in situ* displaying spontaneous $[\text{Ca}^{2+}]_{\text{cyt}}$ oscillations could cause N-methyl-D-aspartate (NMDA) receptor-mediated neuronal excitability. Thus astrocytic spontaneous $[\text{Ca}^{2+}]_{\text{cyt}}$ oscillations lead to glutamate release from these glial cells. Although oscillations were driven by Ca^{2+} released from intracellular stores since they were blocked by SERCA blockers thapsigargin and CPA, they were also inhibited to the similar extent by the dihydropyridine (DHP) antagonist nifedipine (Parri et al., 2001). This demonstrates the involvement of DHP-sensitive L-type VGCCs in astrocytic cytosolic Ca^{2+} dynamics. Conversely, the DHP agonist BayK8644 increased the number of astrocytes displaying cytosolic Ca^{2+} oscillations (Parri and Crunelli, 2003). Additionally, increasing the driving force for Ca^{2+} entry by increasing $[\text{Ca}^{2+}]_{\text{ext}}$ (to 5 mM) increased the number of spontaneously active astrocytes and the number of cytosolic Ca^{2+} transient increases displayed by individual astrocytes (Parri and Crunelli, 2003). Even though the effect of VGCC manipulations on glutamate release from astrocytes of ventrobasal thalamus has not been assessed, it is tempting to speculate that these channels may be a conduit for Ca^{2+} supply for glutamate release from astrocytes (Figure 1).

Ionotropic neurotransmitter receptors represent an additional entry of Ca^{2+} in astrocytes [reviewed in (Verkhratsky, 2009)]. The role of ligand-gated ionotropic receptors in exocytotic glutamate release from astrocytes is provisional at the moment, however. Given that astrocytes can respond to glutamatergic and cholinergic synaptic inputs by increases in $[\text{Ca}^{2+}]_{\text{cyt}}$ (Dani et al., 1992; Porter and McCarthy, 1996; Araque et al., 2002), we briefly discuss nicotinic acetylcholine receptors, and glutamatergic, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA, receptors on astrocytes. Recent findings indicate that astrocytes express functional nicotinic acetylcholine receptors, particularly those with $\alpha 7$ subunits (Sharma and Vijayaraghavan, 2001). Activation of these receptors on astrocytes can lead to $[\text{Ca}^{2+}]_{\text{cyt}}$ increases, which require CICR and the activation of ryanodine/caffeine-sensitive internal stores. The dominant glutamatergic ionotropic receptors on astrocytes are AMPA receptors, which are found in astroglial cells throughout the brain (Verkhratsky and Steinhauser, 2000; Seifert and Steinhauser, 2001; Aguirre et al., 2004). Astrocytes in hippocampus express GluR2 subunit and thus display low Ca^{2+} permeability through their AMPA channels (Seifert et al., 2003). Hence, a challenge of hippocampal astrocytes with AMPA does not cause glutamate release (Bezzi et al., 1998). However, some types of astrocytes do not express the GluR2 subunit. For example, Bergmann glia, an astrocytic cell found in the cerebellum, expresses AMPA receptors that are Ca^{2+} permeable. These cells ensheath the parallel fiber-Purkinje cell synapses (Grosche et al., 1999). There, electrical

stimulation of parallel fibers to release glutamate caused rapid and transient $[Ca^{2+}]_{cyt}$ elevations within Bergmann glial processes (Grosche *et al.*, 1999). Functional astrocytic NMDA receptors were only recently detected in cortical astrocytes (Lalo *et al.*, 2006). Interestingly, astrocytic NMDA channels grossly differ from neuronal since they do not show a Mg^{2+} block at resting membrane potentials (around -80 mV). Whether activation of these, and other ionotropic receptors expressed by astrocytes leading to increases in $[Ca^{2+}]_{cyt}$ would also lead to Ca^{2+} -dependent glutamate release is unknown at the moment.

Another Ca^{2+} entry point that needs further investigation is at plasma membrane Na^+/Ca^{2+} exchangers. While they are known to extrude Ca^{2+} during high cytosolic loads of this cation in conjunction with the plasma membrane Ca^{2+} -ATPase (PMCA), several reports have shown that the exchanger works in reverse to increase $[Ca^{2+}]_{cyt}$ (Goldman *et al.*, 1994; Benz *et al.*, 2004; Rojas *et al.*, 2007). Hence, release of homeocysteic acid (HCA) from cultured astrocytes can be Ca^{2+} -dependent and mediated by activation of glutamate receptors (Benz *et al.*, 2004). It appears that the agonist stimulation led to intracellular Na^+ load through ionotropic glutamate receptors, which then activated Na^+/Ca^{2+} exchangers to extrude Na^+ from astrocytes while importing Ca^{2+} to the cytosol, resulting in a rise of $[Ca^{2+}]_{cyt}$. This cascade of events was inferred from the observation that agonist-induced HCA release was blocked in the presence of benzamil, a Na^+/Ca^{2+} exchanger blocker. While some have argued that the reverse mode of this exchanger only occurs at pathophysiological conditions, it is worth investigating what this exchanger contributes to physiological Ca^{2+} entry and consequential exocytotic glutamate release from astrocytes.

(ii) Mitochondria

In astrocytes, mitochondria form filamentous reticula, and are in perpetual fusion and fission as they are in other cell types (Westermann, 2002). Mitochondria congregate perinuclearly, but can be located at the cell periphery including processes (Collins *et al.*, 2002). To achieve translocation, mitochondria travel on microtubules and actin filaments. While the actin cytoskeleton dictates the distribution of mitochondria, it appears that the intermediate filament vimentin may be involved as well (Tang *et al.*, 2008). Interestingly, in neurons mitochondria display activity-dependent motility at the synapses (Li *et al.*, 2004; Chang *et al.*, 2006; Mironov, 2006).

Mitochondria are efficient Ca^{2+} sinks during periods when rapid increases in $[Ca^{2+}]_{cyt}$ occur in a cell. They possess a mitochondrial Ca^{2+} uniporter (MCU) that can transport Ca^{2+} into the mitochondrial matrix powered by a negative membrane potential (between -140 mV and -180 mV) across the inner mitochondrial membrane generated by the electron transport chain. In contrast to the SERCA and PMCA which have higher affinities for Ca^{2+} than the MCU, Ca^{2+} is taken up by MCU only at $[Ca^{2+}]_{cyt}$ greater than ~ 0.5 μM (Miyata *et al.*, 1991; Simpson and Russell, 1998) and dominates Ca^{2+} transport at ~ 10 μM and above (Alonso *et al.*, 2006). While such high concentrations are normally not reached in the bulk cytosol, they exist at high Ca^{2+} loci known as microdomains (Schneppenburger and Neher, 2005; Oheim *et al.*, 2006). Indeed, Rizzuto *et al.* (1998) showed that mitochondria are juxtaposed to IP_3 channels of the ER and are exposed to higher concentrations of Ca^{2+} than what would exist at the bulk cytosol. Indeed, Ca^{2+} released from the IP_3 -sensitive intracellular stores in mucosal mast cells caused local $[Ca^{2+}]_{cyt}$ increases of over 16 μM (Csordas *et al.*, 2006). Thus, it seems reasonable to assume that similar localized $[Ca^{2+}]_i$ increases would occur in astrocytes.

It should be noted that as conditions in a cell are not homogenous i.e. ion concentration, mitochondrial Ca^{2+} handling is not homogenous either. In T lymphocytes, subsets of mitochondria modulates the entry of Ca^{2+} through the SOC channels by translocation (Hoth *et al.*, 1997; Quintana *et al.*, 2007). This is a likely function of some mitochondria in astrocytes as well. Kolikova *et al.* (2006) demonstrated using total internal reflection fluorescence

microscopy that at least a subset of mitochondria become trapped near the plasma membrane when cultured astrocytes are challenged with glutamate or ATP. Indeed, numerous studies of various cell types report that different subsets of mitochondria take up Ca^{2+} depending whether stimulated cells released ER Ca^{2+} or whether they allowed Ca^{2+} entry from the extracellular space (Simpson and Russell, 1996; Collins et al., 2001).

As sinks to trap free Ca^{2+} released from the ER, mitochondria are positioned to affect transient Ca^{2+} increases required for vesicle fusion, and exocytosis in secretory cells. Reyes and Parpura (2008) demonstrated that mitochondria can modulate $[\text{Ca}^{2+}]_{\text{cyt}}$ and glutamate release in cortical astrocytes challenged with mechanical stimuli. Taking a pharmacological approach, we showed in parallel experiments that blocking the MCU with ruthenium 360 (Ru60) increased the cytosolic Ca^{2+} accumulation, and glutamate release in cortical astrocytes. In contrast to Ru360 treatment, decreasing mitochondrial Ca^{2+} efflux by blocking the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger with CGP37157, or increasing mitochondrial Ca^{2+} load by inhibiting formation of the mitochondrial permeability transition pore with cyclosporin A, decreased cytosolic Ca^{2+} accumulation, and glutamate release in cortical astrocytes. Hence, these data suggest that the transient increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ is correlative to the amount of glutamate released in cortical astrocytes, and that mitochondria have the capacity to modulate the degree of this release. Taken together, astrocytic mitochondria may be active modulators of Ca^{2+} -dependent glutamate release from astrocytes. They could act as temporary holding compartments of excess Ca^{2+} at the fine astrocytic processes surrounding the tripartite synapse, in a similar manner as has previously been shown in neuronal terminals at the neuromuscular junction (Tang and Zucker, 1997).

Concluding Remarks

The purpose of this review was to summarize the Ca^{2+} sources for exocytotic glutamate release from astrocytes. There are three major Ca^{2+} sources that contribute to this process: (i) the predominant source from internal ER stores, (ii) the SOC entry from the extracellular space and (iii) mitochondria, as outlined in Figure 1. There are several issues that remain to be resolved. As indicated earlier, the activation of ionotropic receptors expressed by astrocytes can lead to increases in $[\text{Ca}^{2+}]_{\text{cyt}}$. Whether this excitation is coupled to the Ca^{2+} -dependent exocytotic glutamate release from these cells awaits further experimentation. Furthermore, it will be necessary to determine whether the same Ca^{2+} sources operate under physiological and pathological conditions or whether there are specific Ca^{2+} sources operating under particular conditions. For example, acute oxidative stress in astrocytes causes influx of extracellular Ca^{2+} through L-type VGCCs (Bond and Greenfield, 2007). Whether this Ca^{2+} source plays a role in exocytotic glutamate release from astrocytes and whether such release occurs in various neurodegenerative diseases causatively linked to oxidative stress remains to be assessed. Alternatively, all possible Ca^{2+} sources could operate together in astrocytes at all times, but each contributing a different portion to the total amount of glutamate being released. It appears that addressing these questions in cell culture systems depends on application of sufficient human time and effort, since many of the specific reagents and methods, some of which are described earlier, for teasing apart the contributions from individual Ca^{2+} sources have already been developed. Although such an approach would require a labor intensive effort of information collection and analysis, it is a pre-requisite before taking experiments out of culture dishes and rigorously testing hypotheses on freely-moving animals and humans.

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References

- Aguirre AA, Chittajallu R, Belachew S, Gallo V. NG2-expressing cells in the subventricular zone are type C-like cells and contribute to interneuron generation in the postnatal hippocampus. *J Cell Biol* 2004;165:575–589. [PubMed: 15159421]
- Alonso MT, Villalobos C, Chamero P, Alvarez J, Garcia-Sancho J. Calcium microdomains in mitochondria and nucleus. *Cell Calcium* 2006;40:513–525. [PubMed: 17067669]
- Araque A, Parpura V, Sanzgiri RP, Haydon PG. Glutamate-dependent astrocyte modulation of synaptic transmission between cultured hippocampal neurons. *Eur J Neurosci* 1998a;10:2129–2142. [PubMed: 9753099]
- Araque A, Sanzgiri RP, Parpura V, Haydon PG. Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *J Neurosci* 1998b;18:6822–6829. [PubMed: 9712653]
- Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci* 1999;22:208–215. [PubMed: 10322493]
- Araque A, Martin ED, Perea G, Arellano JJ, Buno W. Synaptically released acetylcholine evokes Ca²⁺ elevations in astrocytes in hippocampal slices. *J Neurosci* 2002;22:2443–2450. [PubMed: 11923408]
- Bass NH, Hess HH, Pope A, Thalheimer C. Quantitative cytoarchitectonic distribution of neurons, glia, and DNA in rat cerebral cortex. *J Comp Neurol* 1971;143:481–490. [PubMed: 4945394]
- Beck A, Nieden RZ, Schneider HP, Deitmer JW. Calcium release from intracellular stores in rodent astrocytes and neurons in situ. *Cell Calcium* 2004;35:47–58. [PubMed: 14670371]
- Benz B, Grima G, Do KQ. Glutamate-induced homocysteic acid release from astrocytes: possible implication in glia-neuron signaling. *Neuroscience* 2004;124:377–386. [PubMed: 14980387]
- Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL, Pozzan T, Volterra A. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* 1998;391:281–285. [PubMed: 9440691]
- Bond CE, Greenfield SA. Multiple cascade effects of oxidative stress on astroglia. *Glia* 2007;55:1348–1361. [PubMed: 17654703]
- Burdakov D, Petersen OH, Verkhratsky A. Intraluminal calcium as a primary regulator of endoplasmic reticulum function. *Cell Calcium* 2005;38:303–310. [PubMed: 16076486]
- Bushong EA, Martone ME, Jones YZ, Ellisman MH. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci* 2002;22:183–192. [PubMed: 11756501]
- Carmignoto G, Pasti L, Pozzan T. On the role of voltage-dependent calcium channels in calcium signaling of astrocytes in situ. *J Neurosci* 1998;18:4637–4645. [PubMed: 9614238]
- Chang DT, Honick AS, Reynolds IJ. Mitochondrial trafficking to synapses in cultured primary cortical neurons. *J Neurosci* 2006;26:7035–7045. [PubMed: 16807333]
- Cholewinski AJ, Hanley MR, Wilkin GP. A phosphoinositide-linked peptide response in astrocytes: evidence for regional heterogeneity. *Neurochem Res* 1988;13:389–394. [PubMed: 2839790]
- Collins TJ, Lipp P, Berridge MJ, Bootman MD. Mitochondrial Ca(2+) uptake depends on the spatial and temporal profile of cytosolic Ca(2+) signals. *J Biol Chem* 2001;276:26411–26420. [PubMed: 11333261]
- Collins TJ, Berridge MJ, Lipp P, Bootman MD. Mitochondria are morphologically and functionally heterogeneous within cells. *Embo J* 2002;21:1616–1627. [PubMed: 11927546]
- Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnoczky G. Structural and functional features and significance of the physical linkage between ER and mitochondria. *J Cell Biol* 2006;174:915–921. [PubMed: 16982799]
- Dani JW, Chernjavsky A, Smith SJ. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron* 1992;8:429–440. [PubMed: 1347996]
- Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci* 2005;8:752–758. [PubMed: 15895084]
- Deitmer, JW.; Singaravelu, K.; Lohr, C. Calcium ion signaling in astrocytes. In: Parpura, V.; Haydon, PG., editors. *Astrocytes in (patho)physiology of the Nervous System*. Boston, MA: Springer; 2008.

- Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron* 2004;43:729–743. [PubMed: 15339653]
- Goldman WF, Yarowsky PJ, Juhaszova M, Krueger BK, Blaustein MP. Sodium/calcium exchange in rat cortical astrocytes. *J Neurosci* 1994;14:5834–5843. [PubMed: 7523629]
- Golovina VA. Visualization of localized store-operated calcium entry in mouse astrocytes. Close proximity to the endoplasmic reticulum. *J Physiol* 2005;564:737–749. [PubMed: 15731184]
- Golovina VA, Blaustein MP. Unloading and refilling of two classes of spatially resolved endoplasmic reticulum Ca(2+) stores in astrocytes. *Glia* 2000;31:15–28. [PubMed: 10816603]
- Grimaldi M, Maratos M, Verma A. Transient receptor potential channel activation causes a novel form of [Ca²⁺] oscillations and is not involved in capacitative Ca²⁺ entry in glial cells. *J Neurosci* 2003;23:4737–4745. [PubMed: 12805313]
- Grosche J, Matyash V, Moller T, Verkhratsky A, Reichenbach A, Kettenmann H. Microdomains for neuron-glia interaction: parallel fiber signaling to Bergmann glial cells. *Nat Neurosci* 1999;2:139–143. [PubMed: 10195197]
- Halassa MM, Haydon PG. The tripartite synapse. In: Parpura, V.; Haydon, PG., editors. *Astrocytes in (patho)physiology of the nervous system*. Boston, MA: Springer; 2009. In Press
- Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG. Synaptic islands defined by the territory of a single astrocyte. *J Neurosci* 2007;27:6473–6477. [PubMed: 17567808]
- Harper JL, Camerini-Otero CS, Li AH, Kim SA, Jacobson KA, Daly JW. Dihydropyridines as inhibitors of capacitative calcium entry in leukemic HL-60 cells. *Biochem Pharmacol* 2003;65:329–338. [PubMed: 12527326]
- Hatton GI. Dynamic neuronal-glia interactions: an overview 20 years later. *Peptides* 2004;25:403–411. [PubMed: 15134863]
- Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev* 2006;86:1009–1031. [PubMed: 16816144]
- Hofmann T, Schaefer M, Schultz G, Gudermann T. Subunit composition of mammalian transient receptor potential channels in living cells. *Proc Natl Acad Sci U S A* 2002;99:7461–7466. [PubMed: 12032305]
- Hoth M, Fanger CM, Lewis RS. Mitochondrial regulation of store-operated calcium signaling in T lymphocytes. *J Cell Biol* 1997;137:633–648. [PubMed: 9151670]
- Hua X, Malarkey EB, Sunjara V, Rosenwald SE, Li WH, Parpura V. Ca²⁺-dependent glutamate release involves two classes of endoplasmic reticulum Ca²⁺ stores in astrocytes. *J Neurosci Res* 2004;76:86–97. [PubMed: 15048932]
- Huang YH, Bergles DE. Glutamate transporters bring competition to the synapse. *Curr Opin Neurobiol* 2004;14:346–352. [PubMed: 15194115]
- Innocenti B, Parpura V, Haydon PG. Imaging extracellular waves of glutamate during calcium signaling in cultured astrocytes. *J Neurosci* 2000;20:1800–1808. [PubMed: 10684881]
- Jeremic A, Jeftinija K, Stevanovic J, Glavaski A, Jeftinija S. ATP stimulates calcium-dependent glutamate release from cultured astrocytes. *J Neurochem* 2001;77:664–675. [PubMed: 11299329]
- Kastritsis CH, Salm AK, McCarthy K. Stimulation of the P2Y purinergic receptor on type 1 astroglia results in inositol phosphate formation and calcium mobilization. *J Neurochem* 1992;58:1277–1284. [PubMed: 1548464]
- Kofuji, P.; Newman, EA. Regulation of potassium by glial cells in the central nervous system. In: Parpura, V.; Haydon, PG., editors. *Astrocytes in (patho)physiology of the nervous system*. Boston, MA: Springer; 2009. In Press
- Kolikova J, Afzalov R, Giniatullina A, Surin A, Giniatullin R, Khiroug L. Calcium-dependent trapping of mitochondria near plasma membrane in stimulated astrocytes. *Brain Cell Biol* 2006;35:75–86. [PubMed: 17940914]
- Lalo U, Pankratov Y, Kirchhoff F, North RA, Verkhratsky A. NMDA receptors mediate neuron-to-glia signaling in mouse cortical astrocytes. *J Neurosci* 2006;26:2673–2683. [PubMed: 16525046]
- Latour I, Hamid J, Beedle AM, Zamponi GW, Macvicar BA. Expression of voltage-gated Ca²⁺ channel subtypes in cultured astrocytes. *Glia* 2003;41:347–353. [PubMed: 12555202]

- Li Z, Okamoto K, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* 2004;119:873–887. [PubMed: 15607982]
- Malarkey EB, Parpura V. Mechanisms of glutamate release from astrocytes. *Neurochem Int* 2008;52:142–154. [PubMed: 17669556]
- Malarkey, EB.; Parpura, V. Mechanisms of transmitter release from astrocytes. In: Parpura, V.; Haydon, PG., editors. *Astrocytes in (patho)physiology of the nervous system*. Boston, MA: Springer; 2009. p. 301-350.
- Malarkey EB, Ni Y, Parpura V. Ca²⁺ entry through TRPC1 channels contributes to intracellular Ca²⁺ dynamics and consequent glutamate release from rat astrocytes. *Glia*. 2008
- Mironov SL. Spontaneous and evoked neuronal activities regulate movements of single neuronal mitochondria. *Synapse* 2006;59:403–411. [PubMed: 16485263]
- Miyata H, Silverman HS, Sollott SJ, Lakatta EG, Stern MD, Hansford RG. Measurement of mitochondrial free Ca²⁺ concentration in living single rat cardiac myocytes. *Am J Physiol* 1991;261:H1123–1134. [PubMed: 1928394]
- Montana V, Ni Y, Sunjara V, Hua X, Parpura V. Vesicular glutamate transporter-dependent glutamate release from astrocytes. *J Neurosci* 2004;24:2633–2642. [PubMed: 15028755]
- Montana V, Malarkey EB, Verderio C, Matteoli M, Parpura V. Vesicular transmitter release from astrocytes. *Glia* 2006;54:700–715. [PubMed: 17006898]
- Mulligan SJ, MacVicar BA. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature* 2004;431:195–199. [PubMed: 15356633]
- Nedergaard M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* 1994;263:1768–1771. [PubMed: 8134839]
- Nedergaard M, Ransom B, Goldman SA. New roles for astrocytes: redefining the functional architecture of the brain. *Trends Neurosci* 2003;26:523–530. [PubMed: 14522144]
- Ni Y, Malarkey EB, Parpura V. Vesicular release of glutamate mediates bidirectional signaling between astrocytes and neurons. *J Neurochem* 2007;103:1273–1284. [PubMed: 17727631]
- Ogata K, Kosaka T. Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience* 2002;113:221–233. [PubMed: 12123700]
- Oheim M, Kirchhoff F, Stuhmer W. Calcium microdomains in regulated exocytosis. *Cell Calcium* 2006;40:423–439. [PubMed: 17067670]
- Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, Oliet SH. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* 2006;125:775–784. [PubMed: 16713567]
- Parpura V, Haydon PG. Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. *Proc Natl Acad Sci U S A* 2000;97:8629–8634. [PubMed: 10900020]
- Parpura V, Basarsky TA, Liu F, Jęftinija K, Jęftinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature* 1994;369:744–747. [PubMed: 7911978]
- Pari HR, Crunelli V. The role of Ca²⁺ in the generation of spontaneous astrocytic Ca²⁺ oscillations. *Neuroscience* 2003;120:979–992. [PubMed: 12927204]
- Pari HR, Gould TM, Crunelli V. Spontaneous astrocytic Ca²⁺ oscillations in situ drive NMDAR-mediated neuronal excitation. *Nat Neurosci* 2001;4:803–812. [PubMed: 11477426]
- Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, Takano H, Moss SJ, McCarthy K, Haydon PG. Astrocytic purinergic signaling coordinates synaptic networks. *Science* 2005;310:113–116. [PubMed: 16210541]
- Perea G, Araque A. Properties of synaptically evoked astrocyte calcium signal reveal synaptic information processing by astrocytes. *J Neurosci* 2005;25:2192–2203. [PubMed: 15745945]
- Perea G, Araque A. Astrocytes potentiate transmitter release at single hippocampal synapses. *Science* 2007;317:1083–1086. [PubMed: 17717185]
- Peuchen S, Clark JB, Duchon MR. Mechanisms of intracellular calcium regulation in adult astrocytes. *Neuroscience* 1996;71:871–883. [PubMed: 8867055]
- Pizzo P, Burgo A, Pozzan T, Fasolato C. Role of capacitative calcium entry on glutamate-induced calcium influx in type-I rat cortical astrocytes. *J Neurochem* 2001;79:98–109. [PubMed: 11595762]

- Porter JT, McCarthy KD. Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. *J Neurosci* 1996;16:5073–5081. [PubMed: 8756437]
- Quintana A, Schwindling C, Wenning AS, Becherer U, Rettig J, Schwarz EC, Hoth M. T cell activation requires mitochondrial translocation to the immunological synapse. *Proc Natl Acad Sci U S A* 2007;104:14418–14423. [PubMed: 17726106]
- Reyes RC, Parpura V. Mitochondria modulate Ca²⁺-dependent glutamate release from rat cortical astrocytes. *J Neurosci* 2008;28:9682–9691. [PubMed: 18815254]
- Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca²⁺ responses. *Science* 1998;280:1763–1766. [PubMed: 9624056]
- Rojas H, Colina C, Ramos M, Benaim G, Jaffe EH, Caputo C, DiPolo R. Na⁺ entry via glutamate transporter activates the reverse Na⁺/Ca²⁺ exchange and triggers Ca⁽ⁱ⁾₂₊-induced Ca²⁺ release in rat cerebellar Type-1 astrocytes. *J Neurochem* 2007;100:1188–1202. [PubMed: 17316398]
- Rychkov G, Barritt GJ. TRPC1 Ca⁽²⁺⁾-permeable channels in animal cells. *Handb Exp Pharmacol* 2007;23–52. [PubMed: 17217049]
- Schneggenburger R, Neher E. Presynaptic calcium and control of vesicle fusion. *Curr Opin Neurobiol* 2005;15:266–274. [PubMed: 15919191]
- Seifert G, Steinhauser C. Ionotropic glutamate receptors in astrocytes. *Prog Brain Res* 2001;132:287–299. [PubMed: 11544996]
- Seifert G, Weber M, Schramm J, Steinhauser C. Changes in splice variant expression and subunit assembly of AMPA receptors during maturation of hippocampal astrocytes. *Mol Cell Neurosci* 2003;22:248–258. [PubMed: 12676534]
- Sharma G, Vijayaraghavan S. Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores. *Proc Natl Acad Sci U S A* 2001;98:4148–4153. [PubMed: 11259680]
- Sherwood CC, Stimpson CD, Raghanti MA, Wildman DE, Uddin M, Grossman LI, Goodman M, Redmond JC, Bonar CJ, Erwin JM, Hof PR. Evolution of increased glia-neuron ratios in the human frontal cortex. *Proc Natl Acad Sci U S A* 2006;103:13606–13611. [PubMed: 16938869]
- Simpson PB, Russell JT. Mitochondria support inositol 1,4,5-trisphosphate-mediated Ca²⁺ waves in cultured oligodendrocytes. *J Biol Chem* 1996;271:33493–33501. [PubMed: 8969213]
- Simpson PB, Russell JT. Role of mitochondrial Ca²⁺ regulation in neuronal and glial cell signalling. *Brain Res Brain Res Rev* 1998;26:72–81. [PubMed: 9600625]
- Simpson PB, Holtzclaw LA, Langley DB, Russell JT. Characterization of ryanodine receptors in oligodendrocytes, type 2 astrocytes, and O-2A progenitors. *J Neurosci Res* 1998;52:468–482. [PubMed: 9589392]
- Sontheimer H, Fernandez-Marques E, Ullrich N, Pappas CA, Waxman SG. Astrocyte Na⁺ channels are required for maintenance of Na⁺/K⁽⁺⁾-ATPase activity. *J Neurosci* 1994;14:2464–2475. [PubMed: 8182422]
- Stephens GJ, Cholewinski AJ, Wilkin GP, Djamgoz MB. Calcium-mobilizing and electrophysiological effects of bradykinin on cortical astrocyte subtypes in culture. *Glia* 1993;9:269–279. [PubMed: 8112820]
- Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE. TRPC1 and TRPC5 form a novel cation channel in mammalian brain. *Neuron* 2001;29:645–655. [PubMed: 11301024]
- Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE. Formation of novel TRPC channels by complex subunit interactions in embryonic brain. *J Biol Chem* 2003;278:39014–39019. [PubMed: 12857742]
- Takemura H, Putney JW Jr. Capacitative calcium entry in parotid acinar cells. *Biochem J* 1989;258:409–412. [PubMed: 2650680]
- Tang HL, Lung HL, Wu KC, Le AH, Tang HM, Fung MC. Vimentin supports mitochondrial morphology and organization. *Biochem J* 2008;410:141–146. [PubMed: 17983357]
- Tang Y, Zucker RS. Mitochondrial involvement in post-tetanic potentiation of synaptic transmission. *Neuron* 1997;18:483–491. [PubMed: 9115741]
- Theodosis DT, Poulain DA, Oliet SH. Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. *Physiol Rev* 2008;88:983–1008. [PubMed: 18626065]

- Tzingounis AV, Wadiche JI. Glutamate transporters: confining runaway excitation by shaping synaptic transmission. *Nat Rev Neurosci* 2007;8:935–947. [PubMed: 17987031]
- Verkhatsky A. Glial calcium signaling in physiology and pathophysiology. *Acta Pharmacol Sin* 2006;27:773–780. [PubMed: 16787559]
- Verkhatsky, A. Neurotransmitter receptors in astrocytes. In: Parpura, V.; Haydon, PG., editors. *Astrocytes in (patho)physiology of the nervous system*. Boston, MA: Springer; 2009. In Press
- Verkhatsky A, Steinhauser C. Ion channels in glial cells. *Brain Res Brain Res Rev* 2000;32:380–412. [PubMed: 10760549]
- Verkhatsky A, Orkand RK, Kettenmann H. Glial calcium: homeostasis and signaling function. *Physiol Rev* 1998;78:99–141. [PubMed: 9457170]
- Wang W, O'Connell B, Dykeman R, Sakai T, Delporte C, Swaim W, Zhu X, Birnbaumer L, Ambudkar IS. Cloning of Trp1beta isoform from rat brain: immunodetection and localization of the endogenous Trp1 protein. *Am J Physiol* 1999;276:C969–979. [PubMed: 10199829]
- Westermann B. Merging mitochondria matters: cellular role and molecular machinery of mitochondrial fusion. *EMBO Rep* 2002;3:527–531. [PubMed: 12052774]
- Yang, Y.; Rothstein, JD. Specialized neurotransmitter transporters in astrocytes. In: Parpura, V.; Haydon, PG., editors. *Astrocytes in (patho)physiology of the nervous system*. Boston, MA: Springer; 2009. In Press
- Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci* 2003;6:43–50. [PubMed: 12469126]

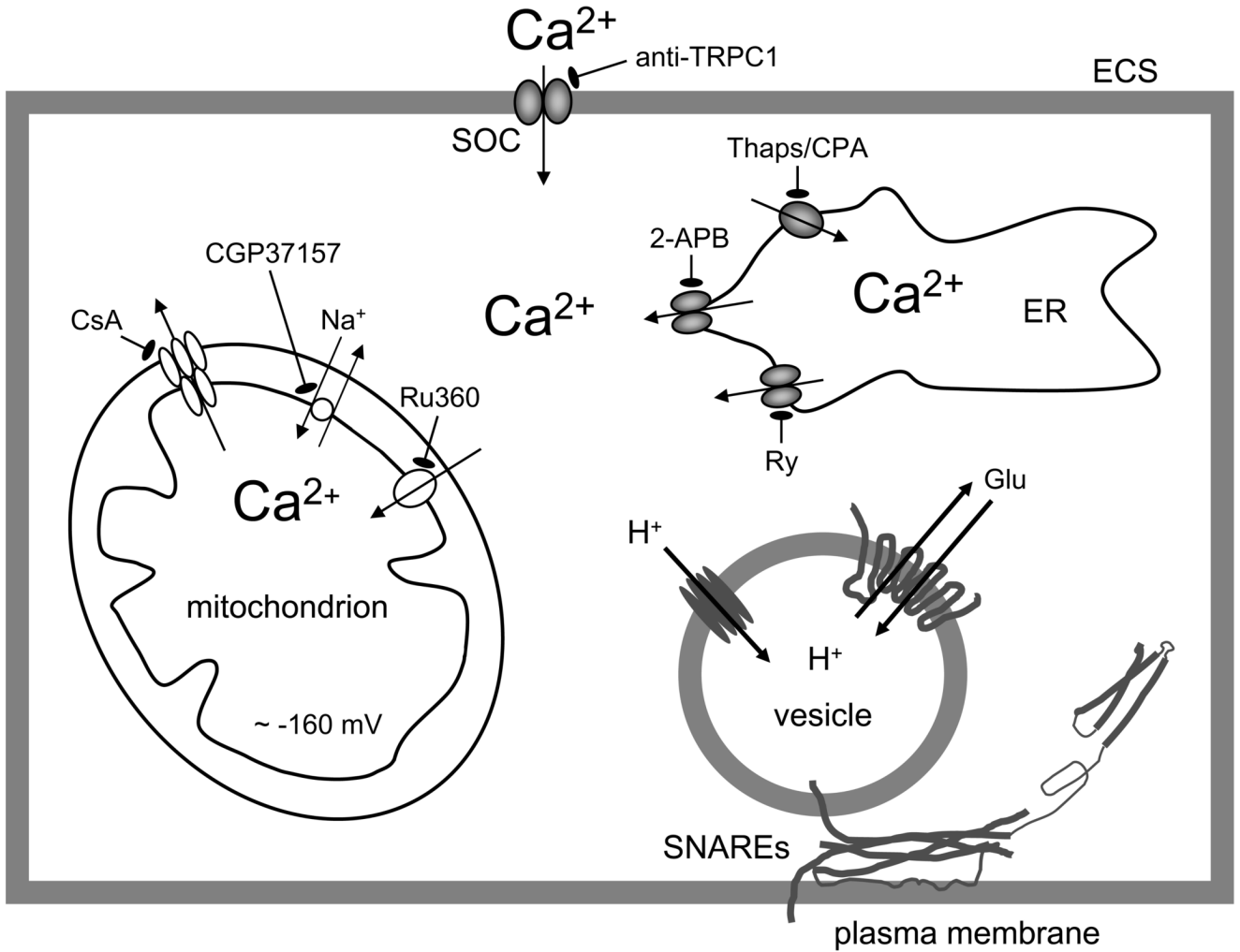


Figure 1. Tripartite Ca^{2+}_{cyt} handling in Ca^{2+} -dependent vesicular glutamate release from astrocytes. A drawing of sources of Ca^{2+} for $[Ca^{2+}]_{cyt}$ increase. $[Ca^{2+}]_{cyt}$ can be affected by the endoplasmic reticulum (ER), store-operated Ca^{2+} (SOC) entry and mitochondria. Ca^{2+}_{cyt} accumulation could be caused by the entry of Ca^{2+} from the internal stores of the ER that possess IP_3 and ryanodine receptors. The pre-incubation of astrocytes with ryanodine (Ry) can block Ca^{2+} release from ryanodine/caffeine-sensitive ER stores, while diphenylboric acid 2-aminoethyl ester (2-APB), an IP_3 R antagonist, reduces Ca^{2+} release from an IP_3 -sensitive stores. Thapsigargin (Thaps) and cyclopiazonic acid (CPA), blockers of store specific Ca^{2+} -ATPase, prevent filling of these stores by Ca^{2+} and, thus, deplete stores of this ion. Ultimately, the (re) filling of ER Ca^{2+} stores requires Ca^{2+} entry from the extracellular space (ECS) through SOC channels, in particular those containing canonical type 1 transient receptor potential (TRPC1) protein. A specific TRPC1 antibody blocks TRPC1 containing SOC channels and reduces Ca^{2+} entry to the cytosol. A negative membrane potential (about -160 mV) exists across the inner mitochondrial membrane. Mitochondrial Ca^{2+} uptake is mediated by the uniporter as the electropotential gradient drives Ca^{2+} into the matrix. Ru360 blocks Ca^{2+} influx through the uniporter. Free Ca^{2+} exits the mitochondrial matrix through the Na^+/Ca^{2+} exchanger, and this process can be blocked by CGP37157. Ca^{2+} accumulation in mitochondria is increased by cyclosporin A (CsA) via preventing formation of the mitochondrial permeability transition

pore, which releases Ca^{2+} and other components of the mitochondrial matrix at high Ca^{2+} loads. Increase of $\text{Ca}^{2+}_{\text{cyt}}$ is sufficient and necessary to cause vesicular fusions and release of glutamate, which requires action by proteins of the ternary SNARE complex and their associated proteins (not shown). Additionally, vesicles possess the vacuolar type H^{+} -ATPase which creates the proton gradient used by vesicular glutamate transporters to fill these organelles with glutamate (Glu). Drawing is not to scale.