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Excitation-Energy Coupling and Vesicle-Based Signaling in Astrocytes

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Abstract. Most of the human brain mass is occupied by the neocortex, which consists of neurons and non-neurons. The latter cells include astrocytes, a heterogeneous glial cell type. While astrocytes have been considered as neuron-subservient entities for almost a century, it is now becoming evident that they are essential in providing homeostatic support to neural networks and that they also actively participate in information processing in the brain. Astrocytes get excited when neurotransmitters bind to their membrane receptors and feed-back by releasing their own signals. This involves vesicles, which store chemicals termed gliotransmitters or more generally gliosignaling molecules. In the former case chemical messengers get released from astrocytic sites proximal to the synapse, which defines communication to occur in the micro-space of contact between the synapse and the astrocyte. In contrast gliosignaling molecules may also be released into the extracellular space. This mode of release resembles the endocrine system. Hence astrocytes are considered to be part of the gliocrine system in the brain, where the glymphatic system mediates the convection of released molecules. This complex system not only plays a role in cell-to-cell communication but also synchronizes the provision of energy for neural networks. Astrocytes contain glycogen, a form of energy store. Excitation of astrocytes by volume transmitters, such as noradrenaline , released by locus coeruleus neurons, activates adrenergic receptors and stimulates glycogenolysis, providing lactate. This chapter briefly reviews how noradrenaline and astrocytes operate to synchronize excitation and energy provision. Moreover, Ca^{2+} -dependent fusion of the vesicle membrane with the plasma membrane in astrocytes is discussed.

Keywords: Astrocyte; Glia; Glycogen; Trafficking; Regulated Exocytosis; Gliotransmitter; Gliosignaling molecules; Antigen presentation

Introduction

Astrocytes are an abundant form of glial cells in the central nervous system (CNS). They are no longer regarded as passive and subservient partners of neurons. In contrast to the established role of providing merely homeostatic metabolic support to neurons, they are active in signaling as well. Since the early 1990s many new functions have been discovered including regulation of synaptogenesis, synaptic transmission, brain microcirculation, roles in the formation and maintenance of the blood–brain barrier (BBB), roles in the integration of adult-born neurons into functional networks and participation in pathologic immune responses and contributions to the formation and resolution of brain oedema (Dong & Benveniste, 2001; Haydon, 2001; Ke et al., 2001; Anderson & Nedergaard, 2003; De Keyser et al., 2003; Nedergaard et al., 2003; Zonta et al., 2003; Abbott et al., 2006; Gordon et al., 2007; Nase et al., 2008; Stevens, 2008; Risher et al., 2009; Sultan et al., 2015). A key process for all these recently discovered functions involves cytoplasmic excitability, a property of astrocytes that respond to stimuli by elevations in secondary messengers, such as cytosolic levels of Ca^{2+} and cAMP (Verkhratsky & Kettenmann, 1996; Verkhratsky et al., 1998; Vardjan & Zorec, 2015). Excited astrocytes are engaged in energy providing processes to metabolically support activated neuronal networks during certain tasks. An important system that controls excitation-energy coupling is the adrenergic system in the CNS, involving the locus coeruleus (LC) neurons, which release catecholamines including noradrenaline (NA) and adrenaline (ADR). This system also consists of target cells such as astrocytes which bear adrenergic receptors (AR) on their plasma membrane and respond with a change in cytosolic levels in D-glucose, a result of activated glycogenolysis (Prebil et al., 2011a). Excited astrocytes communicate their active physiological state to neighbouring cells, where vesicle-based mechanisms play an important role. Exo- and endocytosis, which mediates vesicle-based release are also involved in setting the density of plasma membrane channels, receptors and transporters (Stenovec et al., 2008), determining the quality of the plasma membrane signaling capacity as well as the release of chemical messengers which may affect the function of neighbouring cells in health and disease (Kreft et al., 2004; Osborne et al., 2009; Parpura & Zorec, 2010; Guček et al., 2012; Parpura et al., 2012; Zorec et al., 2012; Potokar et al., 2013b; Vardjan et al., 2015b).

Excitable astrocytes

The hallmark of cytosolic excitability of astrocytes is a transient increase in cytosolic level of Ca^{2+}, a consequence of the activation of various receptors, pumps, and transporters that glial cells express on their plasma membrane and endomembranes, most notably that of the smooth ER, which acts as the dominant intracellular Ca^{2+} store

(Vardjan et al., 2015a). Such a response was first detected by applying the neurotransmitter glutamate to astrocytes (Cornell-Bell et al., 1990b). Not only glutamate, astrocytes detect a myriad of signaling molecules present in the brain parenchyma, some of which are released by astrocytes themselves, and the list of these is getting longer and longer (Verkhratsky et al (2016). When signaling molecules bind to their receptors, this may increase not only cytosolic levels of Ca^{2+} but also other astrocytic cytosolic signals, such as (cAMP) (Vardjan & Zorec, 2015). A prominent stimulus for the increase in cAMP levels are β-adrenergic receptor (β-AR) agonists, which rapidly increase cAMP to a new steady-state level (Vardjan et al., 2014a). However, the increase in cAMP levels are without rapid oscillations as seen in time-dependent changes in levels in cytosolic Ca^{2+} (Vardjan & Zorec, 2015).

Astrocytes express a large number of various types of receptors in culture and in situ, and many of these receptors are metabotropic, high affinity G protein-coupled receptors (GPCRs) (Agulhon et al., 2008; Parpura & Verkhratsky, 2012; Zorec et al., 2012). Depending on the type of the GPCR subunit, distinct cellular responses can be elicited. For example, activation of the Gq subunit leads to the stimulation of phospholipase C (PLC), which hydrolyzes phosphoinositol diphosphate (PIP2) to diacyl-glycerol (DAG) and inositol trisphosphate (IP3). Binding of IP3 to IP3Rs located on the ER (Hua et al., 2004) or even on secretory vesicles (Hur et al., 2010) increases intracellular Ca^{2+} levels through the release of Ca^{2+} from these intracellular organelles. Mitochondria can also act in shaping Ca^{2+} signals, since in astrocytes they can buffer Ca^{2+} through Ca^{2+} uptake and storage (Simpson & Russell, 1998; Reyes & Parpura, 2008). The Ca^{2+} can also enter astrocytes from the extracellular space through voltage-gated Ca^{2+} channels (MacVicar, 1984; Parri & Crunelli, 2001; Latour et al., 2003), ionotropic receptors (Lalo et al., 2011), and the sodium-calcium exchanger (Reyes et al., 2012) as well as through the transient receptor potential canonical type 1-containing channel (Malarkey et al., 2008). However, Gq GPCR activation and release of Ca^{2+} from IP3-sensitive internal stores is the best-accepted mechanism for cytosolic Ca^{2+} increases in astrocytes.

Unlike Gq GPCR activation, stimulation of Gs GPCR subunits in astrocytes triggers adenylyl cyclase (AC) to catalyze the conversion of ATP to cAMP (Rathbone et al., 1991; Vardjan et al., 2014a). This cAMP activates a number of effectors in the cell, primarily cAMP-dependent protein kinase A, which, by phosphorylating cytoplasmic and nuclear targets, mediates many different functional effects. Signaling via cAMP-activated GTP-exchange protein (de Rooij et al., 1998), cAMP-gated ion channels, and Popeye domain-containing proteins (Froese et al., 2012) may also be present (Beavo & Brunton, 2002).

Ca^{2+} excitability in astrocytes has been observed in culture (Cornell-Bell et al., 1990a), in brain slices in situ (Pasti et al., 1997), and in vivo (Hirase et al., 2004) and may occur spontaneously or in response to primary messengers (Cornell-Bell et al., 1990a). Ca^{2+} excitability can propagate from an excited astrocyte to neighbours in the form of intercellular Ca^{2+} waves, which are carried by diffusion of IP3 or Ca^{2+} through gap junctions (Scemes et al., 2000) or via astrocytic release of glutamate or ATP and subsequent receptor-mediated activation of neighboring astrocytes (Bowser & Khakh, 2007). These waves travel 10–20 μm/s (Leybaert & Sanderson, 2012). By contrast, Gs activation induces persistent cAMP elevations (Vardjan et al., 2014a; Vardjan & Zorec, 2015). Whether Gs-induced cAMP excitability can be propagated between neighboring astrocytes (by intercellular cAMP waves), as was observed for Ca^{2+} excitability, needs to be evaluated in the future.

Interestingly, α- and β-ARs coexist on astrocytes and are activated when catecholamines such as NA is increased during activation of the LC. How this event affects astrocyte-based functions related to energy provision is considered next.

Adrenergic system in the CNS: excitation-energy coupling in astrocytes

Most of the energy demand in the brain is linked to electrical and synaptic activity (Attwell & Gibb, 2005), but how energy substrates, needed for ATP synthesis, are delivered to synapses, is still an open question. A simple assumption would be that pyruvate is provided to the mitochondria by glycolysis within the neuron. However, the extensive astrocytic processes in contact with blood vessels are well suited to take up glucose from blood and distribute it, either as glucose itself, or pyruvate or lactate derived from glucose, to astrocytic parts surrounding synapses, possibly by diffusion through gap junctions integrating astroglial syncytia (Rouach et al., 2008). Diffusion of glucose within astrocytes is relatively rapid (Kreft et al., 2013) and may well support glucose delivery via interconnected astrocytes in situ. Although synapses are the main energy consumers in the brain, glycogen, an important CNS energy storage system, is present mainly, if not exclusively, in astrocytes. Many processes, including memory formation,require glycogenolysis (Gibbs et al., 2006; Hertz & Gibbs, 2009), and this appears to be controlled by neuronal release of NA (Gibbs et al., 2010). Thus, NA may be considered as an integrator of excitation and energy provision by astrocytes. In the adult operational (i.e. awake) brain, NA is the main signaling molecule that triggers astroglial Ca^{2+} excitation (Ding et al., 2013).

The primary source of NA in the CNS is LC. Neurons from this nucleus innervate the spinal cord, other regions of the brain stem, the cerebellum, the hypothalamus, the thalamic relay nuclei, the amygdala, the basal nuclei, and the cortex, although some cortical areas are covered by more abundant innervation (Chandler et al., 2014). In all these structures, synchronous activation of LC projections (Bouret & Sara, 2005) leads to coherent and synchronized electrical activity, possibly reflected by gamma waves on an electroencephalogram (Sara, 2015). This may be regarded as a functional "reset" for brain networks (Bouret & Sara, 2005; Sara, 2015), a key process associated with arousal and the sleep–wake cycle, attention and memory, behavioral flexibility, behavioral inhibition and stress, cognitive control, emotions, neuroplasticity, posture, and balance (Benarroch, 2009). In all these functions NA re-

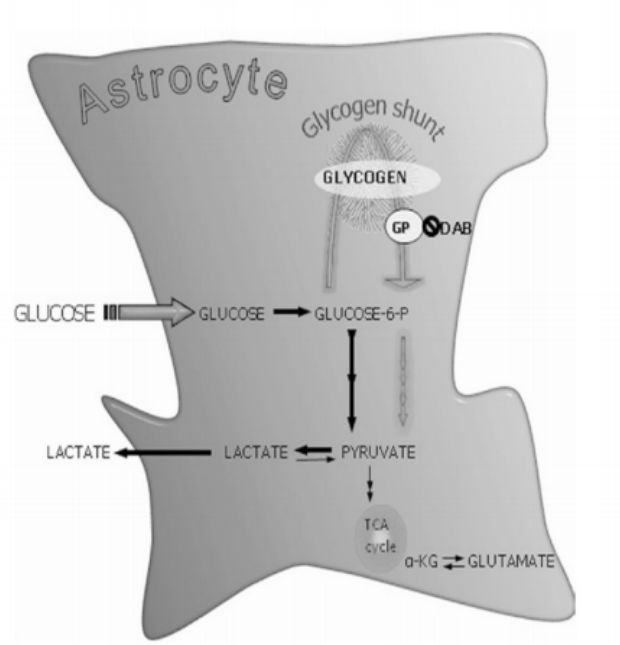

Figure 1. Glucose and glycogen metabolism in astrocytes. Simplified schematic representation of glucose metabolism via glycolysis or via the 'glycogen shunt' illustrating how glucose units may be metabolized via incorporation into and subsequent hydrolysis from the branched glycogen molecule preceding metabolism to pyruvate and lactate, i.e. glycogenolysis. Glucose-6-P, glucose-6-phosphate; TCA, Krebs Cycle in the mitochondrion where α-ketoglutarate (α-KG) is an intermediate for glutamate production in the astrocyte.

leased from LC neurons binds to α- and β-ARs which are present in many cell types including neurons, microglia, and astrocytes. The ARs were among the first receptors to be causally linked to astroglial Ca^{2+} signaling (Salm & McCarthy, 1989; Kirischuk et al., 1996). Astroglial cytosolic Ca^{2+} signals were observed in vivo after stimulation of the LC in anesthetized animals (Bekar et al., 2008). Interestingly in awake animals, stimulation of LC neurons triggered (by activation of β-ARs) widespread astroglial Ca^{2+} signals, which appeared in almost all astrocytes in the field of study (Ding et al., 2013). These observations are consistent with the notion of LC acting as a "reset" for neural networks (Sara, 2015).

In addition to the role of α1-ARs, which mediate the increase in cytosolic Ca^{2+} levels widely in astrocytic networks (Ding et al., 2013), the simultaneous activation of β-ARs leads to the activation of the cAMP-dependent pathway; this in turn instigates rapid degradation of glycogen, which serves as the main energy reserve in the brain (Prebil et al., 2011a; Kreft et al., 2012). Figure 1 depicts glycogen-linked mechanisms in astrocytes and shows how glucose entry through the plasma membrane connects with the glycolytic process via the glycogen shunt, how this is linked to oxidative phosphorylation and the putative synthesis of glutamate and lactate production.

In addition to regulating glucose metabolism, the cAMP signaling pathway also shapes astrocytes; when NA is applied to astrocytes in culture they attain stellate morphology (Vardjan et al., 2014a) and morphological changes in astrocytes are related to memory formation (Ostroff et al., 2014). During pathological states, morphology of astrocytes may change (Olabarria et al., 2010). These morphological changes may be linked to the efficiency of energy provision by astrocytes, since diffusional fluxes of energy-linked molecules are strongly determined by the surface-volume ratio of astrocytic parts (Vardjan et al., 2014b; Vardjan et al., 2014d). Mechanisms that determine astrocyte morphology are unclear, but likely include vesicles interacting with the plasma membrane. Moreover, not only regulating astrocyte morphology, vesicles play an important role in cell-to-cell communication. Next we will discuss this vesicle function in astrocytes.

Versatility of communication by vesicle-based mechanisms

Cytosolic excitability may lead to the release of a variety of chemicals from astrocytes, termed gliotransmitters or more generally gliosignaling molecules. When these molecules interact with neighbouring cells, for example with a synapse, immediately in the vicinity of the astrocytic process, then these messengers are considered gliotransmitters, implying a relatively rapid action, given the micro-space signal confinement, acting in a paracrine or autocrine manner. However, if they get released into the extracellular space and are delivered by convection to distant parts of brain parenchyma via the glymphatic system (Thrane et al., 2014), then the term gliosignaling molecule is more appropriate (Vardjan & Zorec, 2015). Being a gliotransmitter or a gliosignaling molecule both can modulate neuronal excitability (Calegari et al., 1999; Parpura & Verkhratsky, 2012).

For a chemical released from astrocytes to be identified as a gliosignaling molecule, the following criteria are usually taken into account: (i) synthesis by and/or storage in glia; (ii) regulated release triggered by physiological and/or pathologic stimuli; (iii) activation of paracrine or autocrine responses; and (iv) a role in (patho)physiological processes (Parpura & Zorec, 2010; Parpura & Verkhratsky, 2011).

Several mechanisms of gliosignaling molecule release seem to coexist in a single astrocyte (Domingues et al., 2010; Hamilton & Attwell, 2010). In addition to the vesicle-based, non–vesicle-based mechanisms have been identified in glial cells as channels (such as volume-regulated anion channels), connexons/pannexons, hemichannels, ionotropic purinergic receptors and reverse uptake by membrane transporter (see references within (Parpura & Zorec, 2010). For all these mechanisms a concentration gradient is required along which chemical messengers are transported to their targets, however the vesicle-based mechanisms, have certain advantages over the non–vesicle-based modes of release (Guček et al., 2012).

Vesicle-based transmitter and hormone release is based on exocytosis, a process that involves many stages including the merger between the vesicle and the plasma membranes (Coorssen & Zorec, 2012). This universal process, an evolutionary invention of eukaryotic cells (Vardjan et al., 2010), emerged from a prokaryotic-like precursor cell by endosymbiosis (Cooper et al., 2000; Cooper G. M., 2000). Thus, a hallmark of eukaryotic cells, including astrocytes, is a membrane-bound subcellular structure, including mitochondria, Golgi bodies and secretory ves-

icles. When eukaryotic cells evolved 1000–3000 million years ago (Cooper G. M., 2000; Yoon et al., 2004; Spang et al., 2015), this was associated with an increase in cell volume 3 to 4 orders of magnitude. The increased cell size dictated a new organizational set-up. A key reason for this is that signaling and communication within the relatively large volume of a eukaryotic cell could no longer be supported mainly by diffusion-based processes. Hence, subcellular organelles emerged as a solution for the signaling problem in enlarged volume of eukaryotic cells.

Vesicle-based release mechanisms may mediate very rapid or very slow communication between cells. For example, neurotransmitters stored in secretory vesicles can be discharged swiftly through the exocytotic fusion pore that is formed when vesicle membrane merges with the plasma membrane. The probability that a fusion pore is formed swiftly is higher if vesicles are placed adjacent to the plasma membrane, for example, in an active zone. The latency between the signal onset and the response at the postsynaptic membrane in a synapse can be as short as 100 μs (Sabatini & Regehr, 1999).

Vesicle-based mechanism may be utilized when much longer delays are required. For example, the latency can be lengthened by delaying the vesicle delivery to the plasma membrane fusion sites (Potokar et al., 2013b). Moreover, the latency between the stimulus–secretion coupling can be increased by reducing vesicle discharge from the vesicle lumen by regulating vesicle fusion pore kinetics and/or fusion pore diameter (Vardjan et al., 2007). Very long delays between a stimulus and the vesicle merger with the plasma membrane may be achieved, if the stimulus triggers synthesis of the signaling molecules that are released or are incorporated into the plasma membrane. Astrocytes express many plasma membrane receptors, including major histocompatibility complex class II (MHC II), that play a role in antigen presentation, but only when exposed to interferon-γ (IFN-γ). When astrocytes are challenged with IFN-γ, it may take several hours for MHC II molecules to appear in vesicles (Vardjan et al., 2012). Therefore, a delay of a day or more can be achieved by controlling the expression of certain signaling molecules and their vesicle-based delivery to the plasma membrane or even their release from the vesicle lumen. Figure 2 illustrates the requirement of intermediate filaments in this relatively slow mode of vesicle-based signaling in astrocytes. In conclusion, the vesicle-based mechanism is suitable not only for rapid communication, as in neuronal chemical synapses (Sabatini & Regehr, 1999) but also in much longer spanning processes.

The question can be asked whether regulated exocytosis in neurons and in astrocytes exhibits different properties.

Kinetics of regulated exocytosis in astrocytes and in neurons

In principle the mechanism of regulated exocytosis in neurons and in astrocytes is similar, as both cells are eukaryotic cells. When a vesicle membrane fully integrates into the plasma membrane, the area of the plasma membrane increases by the area of the vesicle membrane and this can be monitored elegantly by measuring fluctuations in the plasma membrane capacitance (Cm), a parameter linearly related to the membrane area (Neher & Marty, 1982; Rituper et al., 2013). This technique was used in a number of neuronal and neuroendocrine preparations (Neher, 2012) and in cultured astrocytes (Kreft et al., 2004). The advantage of this approach is that it allows direct testing of the hypothesis that an increase in cytosolic calcium ($[Ca^{2+}]i$), increased by photolysis of caged calcium compounds (Neher & Zucker, 1993), elicits an increase in the whole-cell Cm.

While the Ca^{2+}-induced increase in Cm was blocked in astrocytes by tetanus neurotoxin, indicating that changes in membrane area are associated with a SNARE-dependent vesicular mechanism (Kreft et al., 2004) and a half-maximal response in Cm increase was attained at around 27 μM $[Ca^{2+}]i$, which is similar to the Ca^{2+}-dependency of regulated exocytosis in neurons, recorded by a similar technique (Heidelberger et al., 1994; Bollmann et al., 2000; Kreft et al., 2003), the kinetics of this response in astrocytes was at least two orders of magnitude slower in comparison with the rate of regulated exocytosis recorded by a similar technique in neurons (Kreft et al., 2004). It turns out that in astrocytes regulated exocytosis measured by the afore-mentioned method is the slowest in astrocytes (Neher, 2012; Vardjan et al., 2014c). Hence, astrocytes operate as signal integrators in the CNS; they listen to various signals and respond with a relatively long delay due to the slow responsiveness of the machinery for regulated exocytosis. However, the reason why regulated exocytosis is relatively slow in astrocytes is unknown.

The relatively slow kinetics of glial-regulated exocytosis may be due to many factors, including the slow delivery of vesicles to the plasma membrane and/or distinct (slow) vesicle–plasma membrane fusion mechanisms, perhaps due to a distinct set of molecules. One such molecular distinction between neurons and astrocytes is the absence of synaptotagmin I in astrocytes, which is the key Ca^{2+} sensor in rapid synaptic transmission in neurons (Geppert et al., 1994). What are the Ca^{2+} sensors for regulated exocytosis in astrocytes is still not clear and this is an important topic for future investigation. Vesicle delivery to the target destination may also be a reason for slow regulated exocytosis in astrocytes. Cytoplasmic vesicle mobility over distances of several micrometres was measured in astrocytes and it turned to be tightly regulated, involving cell signaling pathways activated during astrocyte excitability, depending on cytoskeleton dynamics. Under pathologic conditions (ischaemia, trauma, oedema, neuroinflammation), different triggers alter vesicle mobility, as shown by several studies (Potokar et al., 2007; Stenovec et al., 2007a; Potokar et al., 2008; Potokar et al., 2010; Stenovec et al., 2011; Potokar et al., 2012; Trkov et al., 2012; Vardjan et al., 2012; Potokar et al., 2013a; Potokar et al., 2013b) and reviewed by Vardjan et al. (Vardjan et al., 2015b). In addition to vesicle dynamics, relatively slow regulated exocytosis in astrocytes my also be due to relatively slow loading of gliosignaling molecules into vesicles. As pointed out astrocytes are unique in the sense that these cells

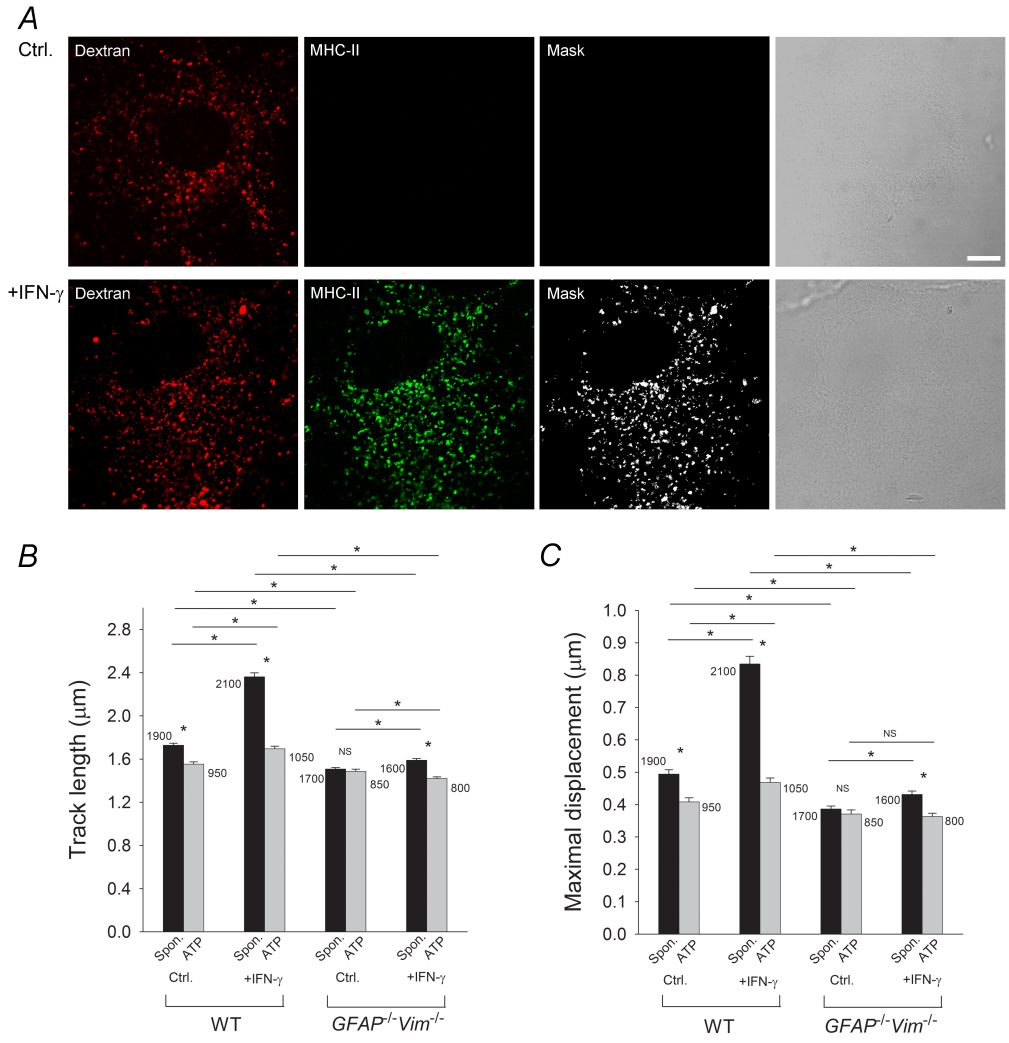

Figure 2. The IFN-γ-induced increase in the mobility of MHC-II compartments in astrocytes is IF dependent. (A) Alexa Fluor® 546-dextran labels MHC-II–positive compartments in IFN-γ-treated WT and GFAP–/–Vim–/– (IF-deficient) primary mouse astrocytes. Fluorescence images of astrocytes labeled with dextran, fixed, and immunostained with antibodies against MHC-II molecules. White pixels (Mask) represent the colocalization mask of green (MHC-II) and red fluorescence pixels (Dextran). Scale bars: 10 μm. (B) Histogram of average vesicle track lengths in control (Ctrl.) and IFN-γ-treated (+IFN-γ) WT and GFAP–/–Vim–/– cells. (C) Histogram of the mean maximal displacements of vesicles in control (Ctrl.) and IFN-γ-treated (+IFN-γ) WT and GFAP–/–Vim–/– cells. The numbers on the bars are the numbers of vesicles analyzed. Values are mean ± SEM. *P < 0.05. Adapted from Vardjan N., Gabrijel M., Potokar M., Svajger U., Kreft M., Jeras M., de Pablo Y., Faiz M., Pekny M., Zorec R. (2012) IFN-γ-induced increase in the mobility of MHC class II compartments in astrocytes depends on intermediate filaments. J Neuroinflammation 9:144; with permission.

are the seat of glycolysis which rapidly generates ATP, but also links to mitochondrial metabolism where glutamate can be synthesized. Therefore, we shall discuss next the vesicle properties of these two gliosignaling molecules.

Vesicle mobility and exocytotic release of glutamate and ATP

A decade ago first spontaneous mobility measurements of membrane-bound vesicles in astrocytes were described (Potokar et al., 2005; Crippa et al., 2006). To determine vesicle mobility, parameters such as the total track length, the path a vesicle travels in a given period of time, the average velocity, the displacement, and the directionality index (ratio between the maximal displacement/total track length) can be measured (see Figure 2). Maximal displacement represents a measure of the maximal net translocation of vesicles (Wacker et al., 1997). Two distinct modes of vesicle mobility have been described in astrocytes: directional (vesicle tracks displaying a straight line, requiring an intact cytoskeleton) and non-directional (vesicle tracks displaying a contorted line), and these modes of mobility were able to switch while a vesicle was being observed (Potokar et al., 2005).

Glutamate-loaded vesicles

Amino acid storing vesicles in astrocytes likely contain glutamate and D-serine (Parpura et al., 1994; Bezzi et al., 2004; Kreft et al., 2004; Montana et al., 2004; Montana et al., 2006; Martineau et al., 2008; Martineau et al., 2013). Glutamate is packaged into vesicles by the vesicular glutamate transporters (VGLUTs) VGLUT1, VGLUT2, and VGLUT3 (Danbolt, 2001; Parpura & Zorec, 2010). Although the existence of VGLUT1 in mouse astrocytes has been questioned (Li et al., 2013), VGLUT1-containing

vesicles in rat astrocytes are small and electron-lucent, with an estimated diameter of ~30 nm in situ (Bezzi et al., 2004) and ~50 nm when they recycle (Stenovec et al., 2007a), but larger sizes have also been reported (Chen et al., 2005; Malarkey & Parpura, 2011). Why different vesicle diameters have been reported for glutamatergic vesicles is not known but this may also be associated with different microscopy techniques used by different investigators. One way to resolve this question in the future would be to use new methods, such as stimulation emission depletion microscopy (STED), a super-resolution fluorescence microscopy technique (Hell & Wichmann, 1994; Jorgacevski et al., 2011) that presents new possibilities to resolve the question of the different diameters reported for vesicles in astrocytes. What is remarkable about the mobility of glutamatergic vesicles is that their spontaneously mobility increases if astrocytes are excited (Stenovec et al., 2007b). These effects were absent in the cells preloaded with high-affinity Ca^{2+} buffer (BAPTA-AM). Microtubules, actin, and vimentin filaments likely play a role in the mobility process of VGLUT1 vesicles, because the disruption of actin attenuated their mobility (Stenovec et al., 2007a). The stimulation-enhanced mobility of glutamatergic vesicles is in contrast to the stimulation-induced attenuation of mobility of peptidergic vesicles, which contain ATP in astrocytes (Pangrsic et al., 2007) and endosomal structures, which likely plays an important role under pathologic conditions (Potokar et al., 2008; Potokar et al., 2010; Potokar et al., 2011; Vardjan et al., 2012).

ATP-loaded vesicles

Extracellular ATP is a chemical messenger (Burnstock, 1995). In astrocytes, it acts as a gliosignaling molecule (Parpura & Zorec, 2010) and a major extracellular messenger for interastrocyte Ca^{2+}-mediated communication (Guthrie et al., 1999; Wang et al., 2000). In addition to non-vesicular modes of ATP release, such as the release of ATP from astrocytes mediated by the connexin hemichannel (Stout et al., 2002; Stehberg et al., 2012) and volume-sensitive organic osmolyte and anion channels (VSOAC) (Blum et al., 2010), Ca^{2+}-dependent exocytotic ATP release from astrocytes has also been confirmed (Parpura & Zorec, 2010). ATP is loaded into vesicles by the VNUT transporter (Sawada et al., 2008; Oya et al., 2013), and was reported to be present in astrocytes (Larsson et al., 2011; Oya et al., 2013). ATP-loaded astrocytic vesicles seem to be heterogeneous. So far, the vesicular distribution of ATP in astrocytes has been shown to overlap with the marker of dense-core granules in the hippocampus, secretogranin II (Calegari et al., 1999; Coco et al., 2003) and with markers of lysosomes (Jaiswal et al., 2007; Zhang et al., 2007; Li et al., 2008; Verderio et al., 2012; Oya et al., 2013). Moreover, it seems to be co-stored together with peptides (Calegari et al., 1999; Belai & Burnstock, 2000; Bodin & Burnstock, 2001; Coco et al., 2003; Pangrsic et al., 2007). In neonatal cortical rat astrocytes, ATP-containing vesicles seem to substantially co-store atrial natriuretic peptide (ANP; 40%) (Pangrsic et al., 2007). Under spontaneous conditions, most of the ATP vesicles were located within 150 nm of the plasma membrane and this coincided with the observation that quinacrine-loaded vesicles (quinacrine is a marker for ATP) displayed mainly non-directional spontaneous mobility and only 4% of vesicles were exhibiting directional mobility. High $[Ca^{2+}]_i$ affected both types of vesicle mobility and completely abolished directional mobility. After a triggered increase in $[Ca^{2+}]_i$, less ATP vesicles were observed in the cells, likely due to Ca^{2+}-activated discharge of the fluorescent cargo by regulated exocytosis. This effect was obstructed by the presence of the dominant-negative soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) domain peptide, which interferes with the formation of the SNARE complex (Zhang et al., 2004; Pangrsic et al., 2007). ATP appears to be also localized in endolysosomes (Jaiswal et al., 2007; Zhang et al., 2007; Li et al., 2008; Verderio et al., 2012; Oya et al., 2013). The mobility properties of these organelles have been described in detail in mouse (Potokar et al., 2010; Vardjan et al., 2012) and rat astrocytes (Stenovec et al., 2011). These vesicles were labelled by LysoTracker dye (Ly) and exhibited slow mobility compared with other vesicle types (Potokar et al., 2013b).

Availability of both glutamate and ATP to be loaded into vesicles depends on the availability of these molecules in the cytoplasm.

Cytoplasmic availability of ATP and glutamate for vesicle-loading

The concentration of ATP and glutamate in the cytoplasm is in the range of millimoles per litre, sufficiently high to support loading into vesicles via specific vesicle transporters, VNUT and VGLUTs, respectively (Danbolt, 2001; Sawada et al., 2008; Parpura & Zorec, 2010). Both ATP and glutamate are associated with the metabolism of glucose, which is linked to glycogen (Figure 1). The glycogen reservoir in astrocytes can provide fuel for energy production during hypoglycaemia via glycogen shunt (Swanson & Choi, 1993; Brown & Ransom, 2007), as well as during normal brain metabolism (Fillenz et al., 1999). Glycolysis and glycogenolysis seem to provide most of the energy required during an abrupt energy demand (Hertz et al., 2007). The glycogen serves as the source of lactate, which may be transferred to neurons (Wender et al., 2000) or converted to pyruvate, which enters the Krebs cycle. When LC neurons are activated, NA may elevate glucose in the cytoplasm of astrocytes fairly rapidly with a time-constant of 100 s (Prebil et al., 2011a). Thus, along with this, one can expect that the rate of ATP and glutamate availability for loading into vesicles may match this time course. It appears that astrocytes respond to NA by being excited, then this drives processes linked to energy production and other processes linked to cell-to-cell communication. Hence astrocytes exhibit mechanisms of excitation-energy coupling which involves vesicle-based mechanisms.

Perpectives

Since the early 1990s the advances in understanding astrocyte function in health and disease has helped to real-

ize that these cells play key homeostatic functions in the brain. In this review, we considered how astrocyte excitability drives, on the one side energy provision and, on the other side, cell-to-cell communication utilizing vesicles, ATP and glutamate. Both molecules are metabolites and gliosignaling entities regulated by adrenergic mechanisms. Thus LC neurons, when they release NA, controls the production/degradation of these two metabolites and their loading into vesicles, which may well be rate limiting for signaling. In the future, these processes will have to be studied by direct measurements of metabolites, such as lactate in the cytoplasm of astrocytes, as was done for glucose (Prebil et al., 2011b). This will bring us closer to understanding how energy support is regulated in view of vesicle dynamics and the vesicular release of gliosignaling molecules, which will help us to understand the spatiotemporal coupling between excitation-energy provision by glia and interactions between neurons and glia.

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Conflict of interest

The authors declare no conflict of interest.

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