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Ionic Signalling in Neuronal-Astroglial Interactions

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Abstract. The name astroglia unifies many non-excitable neural cells that act as primary homeostatic cells in the nervous system. Neuronal activity triggers multiple homeostatic responses of astroglia that include increase in metabolic activity and synthesis of neuronal preferred energy substrate lactate, clearance of neurotransmitters and buffering of extracellular K^+ ions to name but a few. Many (if not all) of astroglial homeostatic responses are controlled by dynamic changes in the cytoplasmic concentration of two cations, Ca^{2+} and Na^+. Intracellular concentration of these ions is tightly controlled by several transporters and can be rapidly affected by activation of respective fluxes through ionic channels or ion exchangers. Here we provide a comprehensive review of astroglial Ca^{2+} and Na^+ signalling.

Keywords: astrocyte; signalling; Ca^{2+} signalling; Na^+ signalling; NCX; sodium-calcium exchanger; cytosolic Na^+.

Astroglial Excitability

Mammalian nervous system consists of electrically excitable neurones that are capable of generation of action potentials and fast chemical (synaptic) transmission and electrically non-excitable neuroglia that control brain homeostasis and defence. These two cell types form complex neuronal-glial networks, witch, by working in concert provide the cellular basis for multiple functions of the nervous system. Neuroglia of the central nervous system (CNS) is generally classified into macroglia (astrocytes and oligodendrocytes) and microglia, which are scions of foetal macrophages invading the neural tissue very early in embryonic development and providing for innate immunity of the CNS.

Astrocytes are arguably the most heterogeneous neuroglial cells; their main role is to maintain homeostasis of the CNS and they indeed do so at all levels from organ to molecules. The term astrocyte (αστρον κψτοσ, - the "star-like" cell in Greek), was coined by Michael von Lenhossek in 1895 (Lenhossek, 1895; Kettenmann & Verkhratsky, 2008) is somewhat misleading because most of these cells in the in vivo brain do not have star-like appearance, displaying a spongioform morphology associated with highly elaborated processes. Astrocytic processes can be subdivided into astroshafts, relatively thick processes containing organelles, and thin flat lamellae, void of organelles, that enwrap synapses (Patrushev et al., 2013). There are many different types of astroglia in the mammalian CNS; these for example include protoplasmic astrocytes of grey matter of the brain and the spinal cord, fibrous astrocytes localised in the white matter, radial Müller retinal glial cells, pseudo-radial cerebellar Bergmann glial cells, velate astrocytes of cerebellum, tanycytes that connect ventricular walls with parts of hypothalamus and spinal cord, pituicytes in the neuro-hypophysis, and perivascular and marginal astrocytes; the human brain and the brain of higher primates also contains interlaminar, polarised and varicose projection astrocytes (Oberheim et al., 2006; Verkhratsky & Toescu, 2006; Kimelberg, 2010; Kimelberg & Nedergaard, 2010; Verkhratsky, 2010; Verkhratsky et al., 2011; Oberheim et al., 2012; Verkhratsky & Butt, 2013).

Astrocytes do not generate action potentials, and yet they are very much capable of perceiving their neurochemical and ionic environment and of mounting specific physiological responses to neuronal activity. Similarly to neurones astrocytes express several classes of plasmalemmal ion channels, including voltage-gated channels, although the densities of Ca^{2+} and Na^+ channels are exceedingly low in vivo rendering current –voltage relationship linear (Verkhratsky & Steinhauser, 2000). However, in cultured astrocytes voltage dependent channels are expressed in higher quantities and regulate intracellular Ca^{2+} dynamics (Yaguchi & Nishizaki, 2010; Letellier et al., 2016). This fact opens a possibility that upon certain physiological or pathological conditions the expression of ion channels in astrocytes may change. In addition astrocytes express full complement of ionotropic and metabotropic receptors to neurotransmitetrs and neurohormones; expression of which however is highly region-specific (Verkhratsky et al., 1998). Astroglial neurotransmitter receptors are activated in response to neuronal activity being an important pathway in the integration of neuronal-glial networks (Verkhratsky, 2010). In contrast to neurones, however, activation of glial receptors does not trigger plasmalemma-associaetd action potentials, but rather induce complex fluctuations in the cytosolic concentrations of major cations, K^+, Na^+ and Ca^{2+}; these ions in turn control astroglial functions and hence represents the specific mechanism of astroglial excitability. Importantly, changes

in cytosolic concentrations of these ions are not restricted to a single cell but rather propagate though glial syncytia. These astroglial syncytia are formed through gap junctions that physically connect adjacent cells.

The gap junctions occur at in between apposing astrocytic membranes of neighbouring cells separated by quite narrow (~ 2 - 2.5 nm) intercellular cleft. The gap junctional permeability is mediated by specialised intercellular channels that protrude membranes of each cell and establish direct contact between cell interiors. These intercellular channels are composed of two precisely aligned connexons each made up from 6 subunits defined as connexins (Dermietzel & Spray, 1993; Dermietzel, 1998). Astrocytes express several types of connexins of which connexins Cx43 dominates and connexins Cx30, Cx40 and Cx45 are expressed to a lesser extent. Connexon channel has a large pore (with a diameter of ~ 1.5 nm), which allows intercellular transfer of ions second messengers [(e.g. inositol 1,4,5 trisphosphate (InsP3)], nucleotides such as ATP and ADP) or metabolic substrates (glucose). As a result the gap junctions form a distinct route for intercellular and long-range signalling which underlie propagating waves of ionic (Ca^{2+}, Na^+ or K^+) signals, or even metabolic waves. Astrocytes use this intracellular signalling route to convey signals over long distances.

In this paper we shall provide a concise overview of astroglial ion signals, molecular mechanisms of their generation and their functional significance.

K^+ Mediated Neuron-Glia Interactions

Although K^+ concentration in the brain extracellular space changes in activity-dependent manner is maintained at a low millimolar range (2.5-5 mM). Excessive increase in extracellular concentration of K^+ ($[K^+]_o$) often reflects a pathological process associated with extreme brain activity or suppression of potassium clearance mechanisms. For example, ischemia leads to intrinsic modifications in Na^+, K^+-ATPase, the major molecule involved in potassium clearance (Jamme et al., 1997a; Jamme et al., 1997b). Elevated extracellular K^+ evokes seizures, spreading depression, migraine and in extreme case cell death. Maintenance of K^+ gradient across plasma membrane is essential for many physiological processes such as membrane potential, repolarization of action potential, neurotransmitters uptake etc. Although global elevations in $[K^+]_o$ are pathological, under physiological conditions $[K^+]_o$ can undergo local and transient changes that can have signalling functions (Shih et al., 2013). Such elevations can occur around axon in association with propagating action potential and in the synaptic cleft during synaptic transmission. Increase in the $[K^+]_o$ within the synaptic cleft can depolarize presynaptic terminal thus changing the probability of glutamate release (Shih et al., 2013). Indeed, presynaptic depolarization is shown to widen arriving action potential that causes enhancement of $[Ca^{2+}]_i$ transient and increases probability of neurotransmitter release (Geiger & Jonas, 2000; Hori & Takahashi, 2009; Sasaki et al., 2011). Elevation of $[K^+]_o$ in the synaptic cleft can also regulate astrocytic glutamate uptake by depolarizing perisynaptic astrocytic processes (PAPs) and affecting voltage-dependent glutamate transporters(Grewer et al., 2008).

What are the sources of K^+ in or around the synapse? General assumption is that is comes as result of K^+ efflux during action potential repolarising phase. However, in glutamate synapses, K^+ can efflux also through postsynaptic AMPA and NMDA receptors(Ge & Duan, 2007). The NMDA receptors are recruited in activity-dependent manner and serve as a major source of K^+ in the synaptic cleft (Shih et al., 2013). Although AMPA receptors responsible for larger peak current than do NMDA receptors, AMPA receptors inactivate very quickly. In contrast, activation of NMDA receptors typically lasts for hundreds of milliseconds (Attwell & Gibb, 2005). Moreover, NMDA receptors have larger single-channel conductance than AMPA receptors (Spruston et al., 1995). Nevertheless, contribution of AMPA receptors increases after induction of long-term potentiation that is associated with insertion of additional postsynaptic AMPA receptors (Ge & Duan, 2007). Moreover, neuronal and astrocytic transporters, ion exchangers and K^+ channels can be involved in regulation of perisynaptic K^+ concentration. For example, K^+/Cl- co-transporter 2 (KCC2) extrudes KCl from the neurons following prolonged neuronal activity when cell depolarization is associated with activation of Cl- permeable GABAA receptors (Rivera et al., 1999). KCC2 mediated extracellular K^+ accumulation depolarises the cells and may serve as a mechanism for their synchronization (Viitanen et al., 2010). Notably, high densities of KCC2 have been found in dendritic spines suggesting its interaction with the other K^+ regulating pathways at the synaptic level synapse (Gulyas et al., 2001).

Neurotransmitters uptake by astrocytes may also contribute to dynamic fluctuations in the $[K^+]_o$. For example, glutamate transporters, exchange glutamate and Na^+ for K^+ and thus can potentially cause local K^+ accumulation (Grewer et al., 2008). Ca^{2+} elevation in astrocytic endfeet activates large-conductance Ca^{2+} dependent potassium channels (BK channels)(Girouard et al., 2010). Because the endfeet touches arterioles, K^+ elevation triggers arteriolar dilation or constriction depending on the concentration. However, K^+ removal is also a major function of astrocytes (Walz, 2000). There are several mechanisms employed by astroglia for extracellular K^+ buffering. Fast K^+ influx through K^+ channels according to electrochemical gradient begins immediately after ambient $[K^+]_o$ increases and shifts K^+ reversal potential towards more depolarising values. This process quickly depolarises astrocytic process ceasing further influx. The rest (and the bulk) of K^+ can be taken into astrocytes by slower energy dependent mechanism represented by Na^+/K^+ ATPase. Spatial buffering of K^+ has also been suggested as the capacity of astrocytes to redistribute locally elevated extracellular K^+ trough gap-junctions that connect these cells to each other (Kofuji & Newman, 2004). Thus, complex interplay between K^+ release mechanisms by neurons and K^+ removal by astrocytes shapes spatio-temporal profile of $[K^+]_o$ dynamics.

Within this profile, extracellular K^+ elevations form various intercellular communication pathways. In glutamate synapses postsynaptic K^+ release serves as retrograde sig-

nal regulating presynaptic release probability and responsible for activity dependent facilitation. K^+ that depolarizes perisynaptic astrocytic lamellae may reduce the efficiency of uptake, and, therefore, glutamate spillover. K^+ efflux due to KCC2 activity synchronises neighbouring neurons, which is important for information processing on the network level. Additionally, K^+ released by astrocytes has a role in cortical neuro-vascular coupling.

Calcium Signalling in Astroglia

Molecular Machinery of Ca^{2+} Signalling

Calcium has been chosen by the evolution as one of the most universal intracellular second messengers, due to its unique qualities (flexible coordination chemistry, high affinity for carboxylate oxygen, which is the most frequent motif in amino acids, rapid binding kinetics) and by its availability in the primordial ocean (Petersen et al., 2005; Case et al., 2007). At high concentrations Ca^{2+} ions cause numerous anti-life effects such as protein and nucleic acid aggregations or precipitation of phosphates; in addition ATP-based energetics require low levels of Ca^{2+} in the cytosol. These factors stipulated the general principle of Ca^{2+} signalling, which is based around steep concentration gradients for Ca^{2+} between the cytosol and the extracellular environment as well as various intracellular compartments. These concentration gradients create electro-driving force for Ca^{2+} aimed at the cytosol where resting Ca^{2+} concentration is kept at level between 50 and 100 nM. Ca^{2+} movements across cellular membranes occur either via diffusion through Ca^{2+} permeable channels or by transport with ATP-consuming pumps or ion-dependent exchangers; the former underlie downhill Ca^{2+} translocation (i.e. in the direction of electro-chemical gradient) whereas the latter provides for up-hill (i.e. against electro-chemical gradient) Ca^{2+} flux. Ca^{2+}-permeable ion channels are represented by several families, which include highly Ca^{2+} selective voltage-gated Ca^{2+} channels, intracellular Ca^{2+} channels (InsP3 receptors or InsP3Rs and ryanodine receptors or RyRs) and Ca^{2+}-release activated Ca^{2+} channels (or CRAC channels, that on a molecular level represent activity of Orai proteins) and cationic channels with various degrees of Ca^{2+} permeability. These cationic channels in turn are represented by ligand-gated channels (or ionotropic neurotransmitter receptors such as for example glutamate, ATP or nicotinic acetylcholine receptors), by extended family of transient receptor potential (TRP) channels and some other types of cationic channels. Ca^{2+} transport against concentration gradients is mainly accomplished by plasmalemmal Ca^{2+} ATPases (PMCAs or plasmalemmal Ca^{2+} pumps), by SarcoEndoplasmic reticulum ATPases (SERCAs or endoplasmic reticulum Ca^{2+} pumps) and by ion exchangers of which the Na^+/Ca^{2+} exchanger or NCX is by far the most important. Inside the cell Ca^{2+} is buffered by Ca^{2+} biding proteins (CBP), affinity of which to Ca^{2+} differs in different cellular compartments. For example, Ca^{2+} affinity of cytosolic CBPs lies in a low nM range, whereas endoplasmic reticulum (ER) CBPs have KD for Ca^{2+} at ~ 0.5 mM. These different affinities determine the range of diffusion of Ca^{2+} ions. In the cytosol CBPs limit diffusion and favour development of local high-Ca^{2+} concentration microdomains, whereas in the ER CBP allow almost free and long-distance Ca^{2+} diffusion that being instrumental for making ER Ca^{2+} tunnels (Solovyova & Verkhratsky, 2003; Petersen & Verkhratsky, 2007). Cellular Ca^{2+} homeostasis is also regulated by mitochondria which are able to accumulate Ca^{2+} (via electrochemically driven diffusion through Ca^{2+} selective channel generally referred to as Ca^{2+} uniporter) and to release Ca^{2+} through mitochondrial Na^+/Ca^{2+} exchanger as well as transient openings of mitochondrial permeability transition pore (Altschuld et al., 1992; Nicholls, 2005).

Effectors of Ca^{2+} signals are Ca^{2+} regulated enzymes (also known as "Ca^{2+} sensors"), biding of Ca^{2+} to which affects functional activity. These Ca^{2+} sensors are many; they have different affinities to Ca^{2+} and are heterogeneously distributed between cellular compartments. These specificities of Ca^{2+} sensors sensitivity to Ca^{2+} and their cellular distribution underlie amplitude and spatial coding of Ca^{2+} signals.

The shape and spatio-temporal organisation of Ca^{2+} signals are defined by the interplay between Ca^{2+} diffusional fluxes and Ca^{2+} transport (Fig. 1). Combinations of those are multiple and labile; as was conceptualised by Michael Berridge, cells can create and rapidly modify "Ca^{2+} signalling toolkits" that adapt Ca^{2+} signalling to the environmental requirements (Berridge et al., 2000; Berridge et al., 2003). Another important feature of Ca^{2+} homeostatic/signalling system is its autoregulation by Ca^{2+} ions themselves as transient changes in Ca^{2+} concentration establish multiple feedbacks modifying the performance of Ca^{2+} handling molecule. As a rule most of Ca^{2+} permeable channels are subject to Ca^{2+}-dependent inactivation, which develops either through direct binding of Ca^{2+} ions to the channel or Ca^{2+}-dependent channel phosphorylation. Similarly, Ca^{2+} pumping by SERCAs is regulated by Ca^{2+} concentration within the ER lumen; this intraluminal Ca^{2+} concentration also controls the availability of intracellular Ca^{2+} channels for activation. Conceptually, lowering Ca^{2+} concentration in the ER facilitates Ca^{2+} uptake and reduces channels activation, whereas increase in intra-ER Ca^{2+} concentration facilitates channels opening and reduces SERCA activity (see (Burdakov et al., 2005; Guerrero-Hernandez et al., 2010) for detailed discussion). Finally Ca^{2+} fluxes are regulated by mitochondria, which by providing ATP and dynamic Ca^{2+} buffering act regulate plasmalemmal Ca^{2+} entry and ER Ca^{2+} uptake (Parekh, 2008; Kopach et al., 2011).

Endoplasmic Reticulum as a Main Source of Astroglial Ca^{2+} Signalling

Astroglial cells respond with intracellular Ca^{2+} elevation to a broad variety of external stimuli from direct mechanical stimulation to a multitude of neurotransmitters, neuromodulators, hormones and other biologically active substances. The ability of astroglia to react with $[Ca^{2+}]_i$ elevation to almost every neuroligands it encounters was firmly established in experiments in cell cultures (Cornell Bell et al., 1990; Charles et al., 1991; Charles et al., 1993; Finkbeiner,

1993; Verkhratsky & Kettenmann, 1996; Verkhratsky et al., 1998). These early experiments were fundamental for glial research because they demonstrated that astrocytes are potentially capable of expressing virtually every receptor modality and that most of these receptors are coupled to ER through the phospholipase C (PLC)/InsP3 signalling cascade. These experiments also highlighted remarkable plasticity of astroglial cells in vitro, as indeed, these cells were able to rapidly modify receptor expression pattern. First studies of astrocytes in situ, in brain slices confirmed the primary importance of $InsP_3$-ER link in generation of astroglial Ca^{2+} signals (Kirischuk et al., 1995; Porter & McCarthy, 1995; Kirischuk et al., 1996; Kirischuk et al., 1999). At the same time these experiments also found that receptor expression in astroglia in neural tissue is restricted to match that of neurons, i.e. immediate neurotransmitter environment (Verkhratsky et al., 1998; Parpura & Verkhratsky, 2012; Verkhratsky et al., 2012; Zorec et al., 2012).

The ER is one of the largest intracellular organelles, which involved in a variety of fundamental cellular processes such as protein synthesis, protein folding and trafficking haulage of secretory products etc. (Baumann & Walz, 2001; Berridge, 2002; Michalak et al., 2002; Verkhratsky, 2005). The ER is also a key organelle of calcium signalling being arguably the largest dynamic Ca^{2+} store able to accumulate, store and release Ca^{2+} ions in response to (patho)physiological stimulation. Ca^{2+} accumulation into the ER lumen is accomplished by SERCA pumping; the Ca^{2+} concentration in the ER at rest (also known as intraluminal Ca^{2+} concentration, $[Ca^{2+}]_L$) is maintained at 0.2 - 1.0 mM range (Mogami et al., 1998; Alonso et al., 1999; Solovyova & Verkhratsky, 2002; Solovyova et al., 2002; Verkhratsky & Petersen, 2002). Release of Ca^{2+} from ER in astroglia is primarily mediated by InsP3 receptors, and their inhibition by pharmacological agents (e.g., heparin) or by genetic deletion often prevents development of Ca^{2+} signals in astrocytes (Kirischuk et al., 1999; Agulhon et al., 2008). Functional role of second type of ER Ca^{2+} release channel, the ryanodine receptor (a Ca^{2+}-gated Ca^{2+} channel) in astroglial Ca^{2+} dynamics remains controversial, although astrocytes do express RyRs both in vitro and in situ (Matyash et al., 2002; Verkhratsky et al., 2002; Beck et al., 2004). The InsP3Rs are simultaneously controlled by InsP3 and Ca^{2+} ions and therefore local increase in $[Ca^{2+}]_i$ facilitates receptor opening thus promoting Ca^{2+}-induced Ca^{2+} release through InsP3Rs. This feature underlies the occurrence of propagating Ca^{2+} waves which in essence represent a wave of ER membrane excitation manifested by propagating recruitment of InsP3 receptors. These Ca^{2+} waves are important for astrocyte physiology; astroglial stimulation usually occurs at the level of distal processes, and Ca^{2+} waves convey this excitation down to the soma. Furthermore, astroglial Ca^{2+} wave travel through astroglial syncytia, being therefore a substrate for astroglial long-range signalling (Giaume & Venance, 1998; Scemes & Giaume, 2006).

Calcium signals produced by activation of ER Ca^{2+} release control many functions of astroglia. In particular ER-originated Ca^{2+} signals are critical for inducing exocytotic release of neurotransmitters (such as for example ATP, glutamate or D-serine) from astrocytes (see (Malarkey & Parpura, 2009; Parpura et al., 2011) for review and references). Inhibition of Ca^{2+} accumulation into the ER by specific blockade of SERCA pumps with thapsigargin that leads to exhaustion of the ER Ca^{2+} content due to an unopposed leak through the endomembrane effectively eliminated Ca^{2+}-dependent release of glutamate from cultured astrocytes (Hua et al., 2004). The same effect was achieved after inhibition of InsP3 receptors by membrane-permeable antagonist diphenylboric acid 2-aminoethyl ester (2-APB), which can also affect the store-operated calcium entry discussed next. The role for ER Ca^{2+} signalling cascade in controlling astroglial release of neuroactive substances was subsequently corroborated in experiments in situ (Fellin et al., 2004; Shigetomi et al., 2008; Henneberger et al., 2010; Rusakov et al., 2011).

Plasmalemmal Ca2+ Influx in Astrocytes: Role of TRP Channels and Ionotropic Receptors

Despite the fact that ER Ca^{2+} store acts as a main source for astroglial Ca^{2+} signalling, astrocytes also possess several mechanisms for Ca^{2+} entry that produce physiologically relevant Ca^{2+} signals. There are little evidence that astrocytes in situ can express functional voltage-gated Ca^{2+} channels, although these channels have been detected in several in vitro experiments (see (Parpura et al., 2011) for detailed review). Two major pathways controlling plasmalemmal Ca^{2+} entry in astroglial cells are represented by store-operated and ligand-operated ion channels.

The store-operated Ca^{2+} entry is generally present in the majority of electrically non-excitable cells. This Ca^{2+} influx pathway (initially described as a "capacitative" Ca^{2+} entry - (Putney, 1986, 1990) is controlled by the Ca^{2+} content in the ER lumen, when decrease in $[Ca^{2+}]_L$ results in the opening of plasmalemmal Ca^{2+}-permeable channels (Parekh & Putney, 2005). Activation of the store-operated Ca^{2+} entry fulfils two functions: first, it provides Ca^{2+} for replenishment of the ER store (the capacitative function), and second, it is important for producing the sustained ("plateau") phase of the Ca^{2+} signal that often outlasts the period of cell stimulation. There are several molecular determinants of the store-operated Ca^{2+} entry. Many types of cells express specific (ICRAC) store-operated channels characterised by extremely high Ca^{2+} selectivity and very low single channel conductance. Activation of these channels reflects interaction of STIM proteins (that detect ER Ca^{2+} concentration) with Orai proteins (Putney, 2007) that form the plasmalemmal channel (these latter proteins were named after Greek gate-keeping goddesses (Feske et al., 2006)). Alternatively store-operated Ca^{2+} influx may involve activation of TRP channels (Smyth et al., 2006).

The store-operated Ca^{2+} entry is functionally expressed in astroglia (Tuschick et al., 1997; Pivneva et al., 2008). At the same time neither I_{CRAC} channels nor STIM/Orai complexes were hitherto detected in astrocytes. In contrast, experimental evidence indicates the role for TRP channels. They are expressed in astrocytes at both mRNA and protein levels, and TRP activity is involved in shaping astroglial Ca^{2+} signals (Pizzo et al., 2001; Grimaldi et

al., 2003; Golovina, 2005). Further analysis revealed that in astrocytes the TRP channels are assembles of brain native heteromultimers (Golovina, 2005; Malarkey et al., 2008) containing obligatory TRPC1 (channel forming subunit) and TRPC4 and/or TRPC5 (auxiliary subunits). Inhibition of TRPC1 channel expression by antisence mRNA or its occlusion with blocking antibody directed at an epitope in the pore forming region of the TRPC1 protein significantly decreased store-operated Ca^{2+} influx in cultured astrocytes (Golovina, 2005; Malarkey et al., 2008) and reduced plateau phase of ATP-activated $[Ca^{2+}]_i$ transients (Malarkey et al., 2008). Likewise, immunological inhibition of TRPC1 protein substantially decreased mechanically-induced Ca^{2+} signalling in astrocytes and suppressed Ca^{2+}-dependent glutamate release (Malarkey et al., 2008).

The second pathway for plasmalemmal Ca^{2+} entry in astrocytes is associated with ionotropic receptors (ligand-gated Ca^{2+}-permeable channels). Several types of ionotropic receptors are present in astrocytes in vitro, in situ and in vivo (see (Verkhratsky & Steinhauser, 2000; Verkhratsky et al., 2009; Lalo et al., 2011b). The most important astroglial ionotropic receptors are α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl D-aspartate (NMDA) glutamate receptors and P2X purinoceptors. Often astroglial AMPA receptors do not express GluR-B (GluR2) subunit, which makes these receptors moderately Ca^{2+} permeable (Muller et al., 1992; Seifert & Steinhauser, 2001). The NMDA receptors identified in cortical astrocytes (Lalo et al., 2006; Verkhratsky & Kirchhoff, 2007; Lalo et al., 2011a; Oliveira et al., 2011) differ in their biophysics and pharmacology from the neuronal ones. In particular, astroglial NMDA receptors are weakly (if at all) sensitive to Mg2+ block at physiological resting potential, and their Ca^{2+} permeability is ~ 2 times lower as compared to neurones (PCa/Pmonovalent ~ 3 vs. ~10 in neurones (Palygin et al., 2010). Nonetheless synaptic actication of astroglial NMDA receptors in cortical slices results in substantial Ca^{2+} signals (Palygin et al., 2010).

Astrocytes also express P2X1/5 and P2X7 purinoceptors, which may create Ca^{2+} fluxes (Lalo et al., 2008; Lalo et al., 2011a; Oliveira et al., 2011). The P2X1/5 have moderate Ca^{2+} permeability ((PCa/Pmonovalent ~ 2), which is sufficient to produce physiologically relevant, Ca^{2+} signals upon appropriate stimulation (Palygin et al., 2010). The P2X7 receptors activation may result in massive Ca^{2+} influx, although this signalling is most likely present only in pathology (Franke et al., 2012).

Sodium Signalling in Astroglia

Dynamic Changes in Cytoplasmic Na^+ Concentration in Astrocytes

At the rest, astrocytes have relatively high cytosolic Na^+ concentration ($[Na^+]_i$); in various astroglial preparations (i.e., in culture and in acute slices) it was determined at ~ 15 - 20 mM (cultured hippocampal astrocytes - 15 - 16 mM (Rose & Ransom, 1996); cultured astrocytes from visual cortex - 17 mM (Reyes et al., 2012); astrocytes in cortical slices - 17 - 20 mM (Unichenko et al., 2012), see also (Kirischuk et al., 2012) for comprehensive review). These levels of resting $[Na^+]_i$ in astrocytes are almost twice higher when compared to neurones (~ 4 - 10 mM see e.g. (Kiedrowski et al., 1994; Rose & Ransom, 1996; Knopfel et al., 1998; Pisani et al., 1998)); high cytosolic Na^+ in astrocytes also has functional consequences because it sets reversal potential for many Na^+-dependent transporters/exchangers, which shall be discussed below.

Stimulation of astrocytes (either mechanical or chemical) induces transient and complex changes in $[Na^+]_i$. For example, application of glutamate to astrocytes in vitro evoked local $[Na^+]_i$ transients and propagating Na^+ waves spreading though astroglial syncytium (Kimelberg et al., 1989; Rose & Ransom, 1997; Bernardinelli et al., 2004). Similarly, both single-cell $[Na^+]_i$ transients and astroglial Na^+ waves were observed in astroglial preparations in situ. In cerebellar Bergmann glia glutamate induced $[Na^+]_i$ increase by 10 - 25 mM above the restring level (Kirischuk et al., 1997, 2007); in hippocampus glutamate induced $[Na^+]_i$ rise and astroglial Na^+ waves (Langer et al., 2012). Astroglial $[Na^+]_i$ in hippocampus was also reported to rise by ~ 7 mM following stimulation with γ-aminobutyric acid (GABA) (Unichenko et al., 2012). Finally, astroglial $[Na^+]_i$ increases are induced by stimulation of synaptic inputs as has been detected in both cerebellum and hippocampus (Kirischuk et al., 2007; Bennay et al., 2008; Langer & Rose, 2009).

Molecular Mechanisms Controlling $[Na^+]_i$ in Astroglia

The cytosolic Na^+ concentration in astrocytes is regulated by Na^+ diffusion through plasmalemmal channels, by Na^+ transport through ATP-dependent pumps and by Na^+ translocation by multiple ion exchangers. Main route for plasmalemmal diffusion of Na^+ across the plasmalemma is associated with ionotropic glutamate and purinoceptors, which produce substantial Na^+ fluxes upon activation. In Bergmann glia, for example, stimulation of AMPA receptors with kainate increases $[Na^+]_i$ by ~ 20 -25 mM (Kirischuk et al., 1997). Sodium can also enter astrocytes through TRP channels, non-specific mechanosensitive cationic channels and possibly through Epithelial Sodium Channel (ENaC)/Degenerin family 21 channels or proton-activated Acid Sensing Ion Channels (ASICs), for review see (Kirischuk et al., 2012). Astrocytes in subfornical organ express specific type of sodium channels sensitive to fluctuations in extracellular Na^+ concentration. These channels (classified as Nax channels) are involved in astroglial chemosensing and regulation of body Na^+ homeostasis (Shimizu et al., 2007). All in all, physiological stimulation of astrocytes trigger substantial Na^+ influx, which is mainly mediated by ionotropic receptors and possibly by TRP channels activated following depletion of ER Ca^{2+} stores.

The sodium-potassium pump or Na^+/K^+ ATPase (NKA) is the main energy-dependent astroglial Na^+ transporter. Astrocytes throughout the CNS express the NKA $\alpha 1/\alpha 2$ subunits. The Na^+/K^+ ATPase is activated following an increase in $[Na^+]_i$ and hence every transient $[Na^+]_i$ elevation promotes Na^+ efflux in exchange for K^+, which may repre-

sent a link between cytosolic Na^+ fluctuations and potassium buffering. Astrocytes are also in possession of multiple ion exchangers or solute carriers (SLC; of which more than 50 families embracing ~ 380 members are known (Hediger et al., 2004; Ren et al., 2007)) that utilise the energy stored in pre-existing ion concentration gradients.

Arguably the most physiologically important is the sodium-calcium exchanger or NCX, which belongs to the SLC8 family (Lytton, 2007). All 3 main isoforms, NCX1, NCX2 and NCX3 are expressed in astroglia. Importantly, the NCX proteins are often concentrated in astroglial perisynaptic processes where they co-localise with NKA and plasma membrane glutamate transporters (Minelli et al., 2007). The NCX can mediate both transport of Na^+ and Ca^{2+} in both directions; generally NCX may operate either in the forward mode (Ca^{2+} extrusion associated with Na^+ influx) or in the reverse mode (Ca^{2+} entry associated with Na^+ extrusion). This is determined by (i) stoichiometry of the exchanger, which is $3Na^+$:$1Ca^{2+}$, (ii) transmembrane concentration gradients for Na^+ and Ca^{2+}, and (iii) the level of membrane potential. High resting $[Na^+]_i$ in astrocytes sets the reversal potential of the NCX ~ -80 mV (see (Kirischuk et al., 2012) for calculations and further details), which is very close to the resting membrane potential. Consequently, the NCX in astrocytes dynamically fluctuates between forward/reverse modes and mediates both Ca^{2+} entry and $[Ca^{2+}]_i$ clearance as well as Na^+ influx/efflux (Kirischuk et al., 1997; Paluzzi et al., 2007; Rojas et al., 2007; Reyes et al., 2012). The reverse mode of the NCX is triggered by mild depolarisation and by Na^+ influx through either ionotropic receptors or neurotransmitter transporters discussed below.

The Na^+-dependent neurotransmitter transporters in astrocytes are mainly represented by plasma membrane transporters for glutamate and GABA. The glutamate transporters, generally classified as the excitatory amino acid (mainly glutamate) transporters 1 to 5 (EAAT1 to EAAT5 belonging to SLC1 family) are fundamental for glutamate homeostasis. Astrocytes, which specifically express EAAT1 and EAAT2 (homologues of which in rodents are known as glutamate transporter 1, or GLT1, and glutamate-aspartate transporter or GLAST, respectively) act as the main sink for glutamate in the CNS accumulating ~80% of glutamate released in the course of synaptic transmission (Danbolt, 2001). Glutamate accumulated into astrocytes is rapidly converted (by another astroglia-specific enzyme glutamine synthetase (Hertz & Zielke, 2004; Olabarria et al., 2011)) into glutamine; the latter is either transported back to neurones where it acts as the major precursor for glutamate and GABA and thus is indispensable for sustained synaptic activity (the glutamate-glutamine or GANA-glutamine shuttles) or is utilised for astroglial energetic (Hertz & Zielke, 2004). The stoichiometry of EAAT1/2 is 1 Glu-:3 Na^+:1K^+:1H^+, of which Na^+, protons and glutamate enter the cell in exchange to K^+ efflux. At the result of this stoichiometry and transmembrane gradients of relevant ions the reversal potential for glutamate transporters is more positive than 50 mV (Kirischuk et al., 2012). This makes the reversal of glutamate transport impossible in physiological conditions; only during strong pathological insults accompanied by massive $[Na^+]_i$ overload and very high extracellular K^+ accumulation the glutamate transport can change direction and provide additional glutamate, which may exacerbate excitotoxicity (Attwell et al., 1993). In physiological conditions activation of glutamate transport in astrocytes triggers inward Na^+ current which can elevate $[Na^+]_i$ by 10 - 20 mM (Kirischuk et al., 2007; Kirischuk et al., 2012). Astrocytes also express GABA transporters of GAT1 and GAT3 types (SLC6 family), which are localised predominantly in astroglial processes surrounding inhibitory synapses. GABA transporters provide for a transmembrane transport of 1 GABA molecule (uncharged in physiological conditions) in exchange for 2 Na^+ ions and 1 Cl- anion. Activation of GABA transporters also result in Na^+ influx that can elevate $[Na^+]_i$ by ~ 7 mM (Unichenko et al., 2012). Importantly the reversal potential for GABA transporters lies very close to astrocytic resting membrane potential and therefore even small elevation in $[Na^+]_i$ can switch the transporter into the reverse mode and hence facilitate GABA release from astrocytes; this release which can inhibit neuronal excitability was indeed detected in cortical slices (Unichenko et al., 2012).

Cellular Na^+ homeostasis is also regulated by mitochondria which are able to accumulate Na^+ through mitochondrial Na^+/Ca^{2+} exchanger (Nicholls, 2005). The NCLX, the solute carrier SLC24A6, is essential molecular component of this exchanger (Palty et al., 2010).

Functional Role of Astroglial Na^+ Signalling

Dynamic fluctuations in cytoplasmic Na^+ concentration can affect surprisingly wide array of molecular targets and cascades that are critical for the homeostatic function of astroglia. First of all, $[Na^+]_i$ modulates homeostasis of several neurotransmitters, that include principal excitatory transmitter glutamate and inhibitory transmitters GABA and glycine. Glutamate uptake is critical for termination of excitatory transmission and as the first step in glutamate-glutamine/GABA glutamine shuttle. Increase in $[Na^+]_i$ decreases the efficacy of glutamate transport; as it were glutamatergic transmission activates Na^+ influx into astrocytes via ionotropic receptors and EAATs. Thus increased $[Na^+]_i]_i$ which coincides with the peak of glutamatergic synaptic transmission event temporarily decreases glutamate uptake, thus transiently increasing the effective glutamate concentration in the synaptic cleft. Levels of $[Na^+]_i$ also influence glutamine synthetase as well as export of glutamine from astrocytes to neurones. The latter is mediated by sodium-coupled neutral amino acid transporter SNAT3/SLC38A3 and is directly controlled by $[Na^+]_i$ (Mackenzie & Erickson, 2004).

Astroglial $[Na^+]_i$ also regulates GABA-ergic transmission through (i) controlling astroglial GABA uptake via GAT1/3 pathway and (ii) by maintaining GABA synthesis in neuronal terminals through supplying glutamine. Astroglial GABA transport system is easy to reverse, because (as mentioned before) its reversal potential is set close to the resting potential of astrocyte. Thus mild depolarisation and even small increases in $[Na^+]_i$ may reverse the

GAT-dependent transport making astrocytes a source of GABA. Additionally, GABA-ergic transmission turned out very sensitive to astroglial glutamine supply, and inhibition of glutamine synthetase substantially suppresses GABA-ergic inhibitory transmission (Ortinski et al., 2010). Similarly astroglial $[Na^+]_i$ regulates the efficacy of glycine clearance from the relevant synapses.

Dynamic changes in astroglial $[Na^+]_i$ modulate Ca^{2+} signalling by defining the mode of operation of NCX. Increase in $[Na^+]_i$ were shown to induce additional Ca^{2+} influx that contributed to neurotransmitter-evoked $[Ca^{2+}]_i$ transients (Kirischuk et al., 1997). Such calcium entry through NCX was even demonstrated to induce exocytotic release of neurotransmitters from astroglia (Benz et al., 2004; Paluzzi et al., 2007; Reyes et al., 2012).

Astroglial Na^+ signals are coupled to several important homeostatic pathways. In particular $[Na^+]_i$ levels directly control the activity of NKA and $Na^+/K^+/Cl^-$ co-transporter NKCC1 thus regulating K^+ buffering. The $[Na^+]_i$ controls the activity of sodium-proton exchanger and sodium-bicarbonate transporter, both being critical for pH homeostasis (see (Kirischuk et al., 2012) for further discussion).

Finally, $[Na^+]_i$ controls on of the most fundamental astroglial functions - that is the metabolic support of neurones. The latter occurs in the form of astrocyte-neurone lactate shuttle, when astrocytes supply active neurones with their preferred energy substrate lactate (Belanger et al., 2011; Suzuki et al., 2011; Pellerin & Magistretti, 2012). Neuronal-activity induced elevation of astroglial $[Na^+]_i$ triggers lactate synthesis mediated through NKA; and therefore astroglial Na^+ signalling is fundamental for neuronal metabolic support.

Concluding Remarks

Rapid astroglial signalling that is fundamental for neuronal-glial communications is mediated through fluctuations of cytoplasmic concentrations of two principal cations calcium and sodium. Neuronal activity triggers complex and highly organised in both temporal and spatial domains changes in $[Ca^{2+}]_i$ and $[Na^+]_i$, which in turn regulate multiple effector pathways that control homeostatic function of astroglia.

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