Sodium Fluxes and Astroglial Function

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Abstract

Astrocytes exhibit their excitability based on variations in cytosolic Ca^{2+} levels, which leads to variety of signalling events. Only recently, however, intracellular fluctuations of more abundant cation Na^+ are brought in the limelight of glial signalling. Indeed, astrocytes possess several plasmalemmal molecular entities that allow rapid transport of Na^+ across the plasma membrane: (1) ionotropic receptors, (2) canonical transient receptor potential cation channels, (3) neurotransmitter transporters and (4) sodium-calcium exchanger. Concerted action of these molecules in controlling cytosolic Na^+ may complement Ca^{2+} signalling to provide basis for complex bidirectional astrocyte-neurone communication at the tripartite synapse.

Keywords

Ionotropic receptors • Sodium-calcium exchanger • Sodium potassium pump • Glutamate transporter • Sodium signalling

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25.1 Introduction

Neuroglia represent the main cellular homeostatic system of the brain. Evolution of the central nervous system (CNS) resulted in a high specialisation of elements of brain circuitry: neurones perfected rapidly propagating action potentials and synaptic transmission, whereas glial cells assumed full responsibility for brain homeostasis and defence. Astrocytes, which are the main type of glial cells in the brain and in the spinal cord, have an astonishingly wide array of functions that include regulation of neurogenesis and CNS development, shaping the brain micro-architecture, controlling ion and neurotransmitter homeostasis in the extracellular space, providing activity-dependent metabolic support to neurones and mounting the evolutionary conserved astrogliotic response to CNS lesions (for general review of astroglia in physiology and pathophysiology, see (Heneka et al. 2010; Kettenmann and Ransom 2005; Kimelberg and Nedergaard 2010; Nedergaard et al. 2010; Oberheim et al. 2006; Rodriguez et al. 2009; Verkhratsky 2009, 2011; Verkhratsky and Butt 2007; Verkhratsky et al. 2011)).

Astrocytes are in a possession of several signalling cascades that are activated in response to various physiological and pathological stimuli. These signalling cascades are triggered by activation of numerous plasmalemmal metabotropic and ionotropic receptors (Lalo et al. 2011b; Verkhratsky et al. 2009; Verkhratsky and Steinhauser 2000). The calcium signalling system is of a particular importance for electrically non-excitable astroglial cells, and propagating waves of inositol 1,4,5 trisphosphate ($InsP_3$)-mediated Ca^{2+} release from the endoplasmic reticulum store are considered to be a specific form of astroglial excitability (reviewed in (Agulhon et al. 2008; Parpura et al. 2011; Verkhratsky 2006; Verkhratsky et al. 1998)). Glial calcium signals, however, are rather slow when compared to the speed of synaptic transmission and may not necessarily participate in local neuronal-glial signalling at the level of individual synapses. In the CNS, a substantial proportion of synaptic contacts is closely enwrapped by astroglial membranes (Peters et al. 1991) which effectively shield the synapses preventing neurotransmitter spillover, which ascertains spatial precision of synaptic signalling. In addition to forming physical barrier, perisynaptic processes of astrocytes are endowed with neurotransmitter transporters that provide for neurotransmitter removal and neurotransmitter homeostasis thus contributing to functional isolation of individual synapses. The perisynaptic processes are also rich in ionotropic receptors, cationic channels and sodium-dependent pumps (Na^+/K^+ pump, Na^+/HCO_3^- co-transporters, etc.), the latter being critical for maintaining ion homeostasis in the synaptic cleft.

In this chapter, we shall overview pathways governing sodium fluxes through astroglial plasma membrane and critically address the question of the importance of local sodium fluctuations in the function of astroglial cell. We shall focus on several plasmalemmal Na^+-transporting systems that include ionotropic receptors, canonical transient receptor potential (TRPC) cation channels, Na^+/Ca^{2+} exchanger and Na^+-dependent glutamate transporters.

25.2 Ionotropic Receptors

Astroglial cells are highly heterogeneous in their morphology and function; astrocytes from different brain regions also differ in their assortment of neurotransmitter receptors (Matyash and Kettenmann 2010; Verkhratsky 2011; Verkhratsky and Steinhauser 2000). Astroglial ionotropic receptors are generally represented by receptors for glutamate and adenosine 5′-triphosphate (ATP). Ionotropic glutamate receptors of α-amino-3-hydroxy-5-methyl-isoxazole propionate (AMPA) type are present in astrocytes throughout the CNS, including hippocampus, cerebellum and cortex (Condorelli et al. 1999; Gallo and Ghiani 2000; Seifert and Steinhauser 2001; Steinhäuser and Gallo 1996; Verkhratsky and Kirchhoff 2007a). All four subunits of AMPA receptors have been detected in astroglia, although the actual assembly varies between brain regions. In hippocampal astrocytes, the glutamate receptor (GluR)2 and GluR4 subunits

are predominantly expressed, which stipulates specific electrophysiology (linear *I-V* relation and low Ca^{2+} permeability)(Gallo and Ghiani 2000; Seifert and Steinhauser 1995; Steinhäuser and Gallo 1996); in cortical astroglial cells, the GluR1 and GluR4 subunits are the most abundant (Conti et al. 1994). In Bergmann glial cells in situ and in several in vitro astroglial preparations (Geiger et al. 1995; Muller et al. 1992), the AMPA receptors are devoid of GluR2 subunit that makes the receptors moderately permeable to Ca^{2+} ($P_{Ca}/P_{monovalent} \sim 1–1.5$ (Burnashev et al. 1996; Isa et al. 1996; Itazawa et al. 1997; Pankratov et al. 2009)). Astroglial N-methyl D-aspartate (NMDA) receptors were characterised both in vitro and in situ (Kondoh et al. 2001; Lopez et al. 1997; Nishizaki et al. 1999; Puro et al. 1996), in particular, in astroglial cells from cortex and spinal cord (Lalo et al. 2006; Schipke et al. 2001; Verkhratsky and Kirchhoff 2007b; Ziak et al. 1998). Both NMDA receptor-specific mRNAs and receptor protein were found in cortical astrocytes (Conti et al. 1996; Schipke et al. 2001). In the cortex, the NMDA-mediated astroglial currents are positively potentiated by glycine and are blocked by NMDA antagonists D-2-amino-phosphonopentanoic acid and MK-801 (Lalo et al. 2006; Palygin et al. 2011). The astroglial NMDA receptors have several peculiar features (Lalo et al. 2006; Palygin et al. 2010) which include weak Mg^{2+} block at characteristic levels of astroglial membrane potential of −80 mV (the block develops at V_m values ~ −100 to −120 mV) and moderate Ca^{2+} permeability ($P_{Ca}/P_{monovalent} \sim 3$). Incidentally, similar Mg^{2+} sensitivity was determined in oligodendroglial NMDA receptors (Karadottir et al. 2005; Micu et al. 2006; Salter and Fern 2005), which possibly allows classifying a special class of glial NMDA receptors. Based on electrophysiology, Ca^{2+} permeability and sensitivity to NR2C/D subunit-selective antagonist UBP141 the most probable assembly of glial NMDA receptors include two NR1, one NR2C/D and one NR3 subunit (Palygin et al. 2011).

Astroglial functional expression of ionotropic ATP (P2X) receptors remains poorly characterised. The mRNAs specific for various P2X receptors subunits were identified in cultured astrocytes, in freshly isolated retinal Müller cells and in astrocytes in situ (Franke et al. 2001, 2004; Fumagalli et al. 2003; Jabs et al. 2000; Lalo et al. 2008). At the protein level (as determined by immunoreactivity), $P2X_2$, $P2X_3$ and $P2X_4$ receptors were identified in astrocytes from the nucleus accumbens (Franke et al. 2001); the $P2X_1$ and $P2X_2$ receptors were found in astroglial cells in the cerebellum and in the spinal cord (Kanjhan et al. 1996; Loesch and Burnstock 1998). Immunoreactivity for $P2X_4$ receptors was detected in astrocytes from the brainstem (Ashour and Deuchars 2004). In the hippocampus, immunostaining revealed astroglial expression of $P2X_{1–4}$, $P2X_6$ and $P2X_7$ subunits (Kukley et al. 2001).

Functionally $P2X_{1/5}$ heteromeric receptor-mediated currents were identified in cortical astrocytes (Lalo et al. 2008, 2011c). These $P2X_{1/5}$ heteromeric receptors are characterised by special features which include (1) a very high sensitivity to ATP (EC_{50} for current activation of ~40 nM), (2) biphasic kinetics with distinct peak and steady-state components and (3) very little desensitisation in response to the repetitive agonist applications. As a result, the $P2X_{1/5}$ receptors allow cortical astrocytes to detect extremely low levels of extracellular ATP. Astroglial $P2X_{1/5}$ receptors have a moderate Ca^{2+} permeability ($P_{Ca}/P_{monovalent} \sim 2$ (Palygin et al. 2010)). The $P2X_7$ receptor-mediated currents were also detected in cortical astrocytes in situ (Oliveira et al. 2011), although their low sensitivity to the ATP possibly indicates their pathophysiological importance (Illes et al. 2011). P2X receptor(s)-mediated Ca^{2+} signalling was also described in astroglial cells from acutely isolated optic nerves. These Ca^{2+} signals were inhibited by P2X receptor antagonist NF023 (James and Butt 2001); in addition, astrocytes from the optic nerve seem to express functional $P2X_7$ receptors (Hamilton et al. 2008).

Astroglial ionotropic receptors are activated by endogenous neurotransmitters released in the course of synaptic transmission. In the cortical astrocytes voltage-clamped in the brain slice, both NMDA and $P2X_{1/5}$ receptors mediated the major part of currents triggered by electrical

stimulation of neuronal afferents (Lalo et al. 2006, 2011a). The spontaneous ('miniature') currents mediated by AMPA/NMDA glutamate receptors and $P2X_{1/5}$ receptors were also detected in cortical astrocytes indicating close apposition of astroglial membranes bearing these receptors to the presynaptic sites of neurotransmitter release (Lalo et al. 2006, 2011a, b).

Taken together, astrocytes have several types of fast ionotropic receptors, activated by neurotransmitters released to the synaptic cleft. All these receptors, however, have relatively low Ca^{2+} permeability with predicted fractional Ca^{2+} currents in the range of 1–5 %. At the same time, activation of these receptors at resting membrane potential triggers currents mainly carried by Na^+ ions.

25.3 TRP Cationic Channels

The detailed analysis of various types of cationic channels expressed in astroglia is still needed. Nonetheless, these channels are potentially important for controlling cytosolic sodium concentration because negative resting potential of astrocytes makes Na^+ virtually the sole permeating cation. Among many cationic channels, the products of TRP genes have been identified in astrocytes (Golovina 2005; Grimaldi et al. 2003; Malarkey et al. 2008; Pizzo et al. 2001). These TRP channels are reported to be activated following intracellular Ca^{2+} release acting as store-operated channels (Parpura et al. 2011). It was shown that antisense-based inhibition of expression of the TRPC1 gene (Golovina 2005) or occlusion of the same channel by blocking antibodies raised against the TRPC1 protein channel pore (Malarkey et al. 2008) markedly inhibited store-operated Ca^{2+} entry in cultured astrocytes. In addition to TRPC1, acutely isolated astrocytes as well as astrocytes in vitro express TRPC4 and TRPC5 subunits which are needed to form functional TRPC channel (Strubing et al. 2001, 2003). The Na^+ fluxes generated by activation of TRPC channels have not yet been characterised; nonetheless, it is conceivable to speculate that metabotropically induced depletion of the ER Ca^{2+} stores results not only in $[Ca^{2+}]_i$ signalling but also in elevation of $[Na^+]_i$ through the opening of store-operated TRPC channels.

25.4 Neurotransmitter Transporters

Astroglia is central for neurotransmitter homeostasis, turnover and metabolism in the CNS (Danbolt 2001; Verkhratsky and Butt 2007). The action of two most important transmitters in the brain, glutamate and γ–aminobutyric acid (GABA), critically depends on astroglial transporters that remove these transmitters from the cleft thus terminating their action. The subsequent astroglial processing of glutamate through glutamine-glutamate shuttle is fundamental for replenishing glutamatergic terminals, which are incapable of producing glutamate from their own resources (Hertz and Zielke 2004). Glutamate and GABA transport into astrocytes is achieved through Na^+-dependent transporters that utilise energy of transmembrane Na^+ gradient. Astroglial glutamate transporters are represented by excitatory amino acid transporter type 1 and 2 (EAAT1 and EAAT2; analogues of these transporters in rodents are known as glutamate/aspartate transporter, GLAST and glutamate transporter-1, GLT-1 (Danbolt 2001; Gadea and Lopez-Colome 2001)). The stoichiometry of transporting one molecule of glutamate through both transporters involves influx of three Na^+ ions and one H^+ ion and efflux of one K^+ ion (Owe et al. 2006; Zerangue and Kavanaugh 1996). As a result, the transporter generates inward cationic current and produces substantial elevation of cytosolic Na^+ concentration (Kirischuk et al. 2007). The GABA transporters expressed in astrocytes (GAT1-3, (Heja et al. 2009)) are similarly Na^+ dependent with a stoichiometry of $2Na^+$/1GABA.

25.5 Sodium-Calcium Exchanger

Astrocytes express all three types of mammalian Na^+/Ca^{2+} exchangers, namely, NCX1, NCX2 and NCX3, which are primarily localised in perisynaptic processes, in particular those associated with excitatory synapses (Minelli et al. 2007). According to their thermodynamics (NCX

stoichiometry is $3Na^+/1Ca^{2+}$), the NCX may operate in both forward (Ca^{2+} extrusion associated with Na^+ influx) and reverse (Ca^{2+} entry associated with Na^+ extrusion) modes. The transition between forward/reverse operations is controlled by transmembrane ion gradients and the level of membrane potential (DiPolo and Beauge 1983). Both modes of NCX activity are present in astroglial cells in vitro and in situ through analysing respective $[Ca^{2+}]_i/[Na^+]_i$ concentrations (Goldman et al. 1994; Kirischuk et al. 1997; Matsuda et al. 1996; Takuma et al. 1994). The NCX dynamically fluctuates between forward/reverse modes; in Bergmann glial cells, the NCX working in reverse mode significantly contributes to the peak $[Ca^{2+}]_i$ elevation following activation of kainate receptors; at the same time, NCX participates in relaxation of kainate-mediated $[Ca^{2+}]_i$ transients by extruding Ca^{2+} in the forward mode (Kirischuk et al. 1997). The reverse mode of NCX is activated following Na^+ entry via glutamate transporter in cultured cerebellar astrocytes (Rojas et al. 2007). Similarly, mild depolarization induced by high extracellular K^+ stimulation of adult rat astrocytes in culture promoted reverse mode of NCX that generated $[Ca^{2+}]_i$ transients (Paluzzi et al. 2007). These NCX-associated transients were specifically blocked by 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiourea (KB-R7943), a drug selectively inhibiting reverse mode of NCX operation (Paluzzi et al. 2007). Treatment of unstimulated cultured astrocytes with KB-R7943 also cased moderate decreases in the resting $[Ca^{2+}]_i$ suggesting that NCX may operate in reverse mode at rest (Reyes et al. 2011). This seems to be a plausible suggestion because reversal potential for NCX calculated from the $[Ca^{2+}]_i$ and $[Na^+]_i$ levels measured from these cells was −98 mV. The resting potential of these cultured astrocytes is ~ −70 mV which should set the resting operation mode of NCX as the reversed one.

25.6 Sodium Dynamics in Astrocytes

The resting intracellular Na^+ concentration in astrocytes is generally somewhat higher that in neurones, being ~ 10 mM in cultured cortical astrocytes (Chatton et al. 2003; Floyd et al. 2005), 15–16 mM in cultured hippocampal astrocytes (Rose and Ransom 1996a), 17 mM in cultured astrocytes from visual cortex (Reyes et al. 2011) and ~20 mM in astrocytes in situ in cortical slices (Kirischuk, unpublished observations). In neurones in contrast, average $[Na^+]_i$ is substantially lower being determined at 4 mM in cultured cerebellar granular cells (Kiedrowski et al. 1994), 9 mM in cultured hippocampal neurones (Rose and Ransom 1996a), ~11 mM in dopaminergic cells in substantia nigra pars compacta (Knopfel et al. 1998) and 10 mM in pyramidal neurones from cortical slices (Pisani et al. 1998). Chemical stimulation of astrocytes triggers spatio-temporally organised $[Na^+]_i$ fluctuations. Exposure of cultured astrocytes to glutamate triggered both $[Na^+]_i$ transients and propagating $[Na^+]_i$ waves (Bernardinelli et al. 2004; Kimelberg et al. 1989; Rose and Ransom 1996b, 1997). Similarly, $[Na^+]_i$ transients occur in situ in Bergmann glial cells and hippocampal astrocytes exposed to exogenous ionotropic glutamate receptor agonists or to electrical stimulation of neuronal afferents (Bennay et al. 2008; Kirischuk et al. 1997, 2007). Stimulation of ionotropic glutamate receptors can elevate $[Na^+]_i$ by 10–25 mM, (Fig. 25.1a) (Deitmer and Rose 2010; Kirischuk et al. 2007). In addition, extracellular glutamate activates glutamate transporters, which also produce substantial Na^+ fluxes elevating $[Na^+]_i$ by 10–20 mM (Fig. 25.1b (Kirischuk et al. 2007)). There are indications that Na^+ can travel between astrocytes via gap junctions, and inhibition of the latter desynchronises $[Na^+]_i$ dynamics in astroglia (Bernardinelli et al. 2004; Rose and Ransom 1996a). Astroglial $[Na^+]_i$ signals are also triggered by stimulation of neuronal afferents; these $[Na^+]_i$ responses develop in parallel with glial synaptic currents mediated by both ionotropic receptors and glutamate transporter (Bennay et al. 2008; Clark and Barbour 1997; Kirischuk et al. 2007). Short bursts of stimuli (5–10 pulses) elevated $[Na^+]_i$ by 5–10 mM. These $[Na^+]_i$ transients last much longer than glutamate-induced $[Ca^{2+}]_i$ responses; the decay time constant of $[Na^+]_i$ transients is about 100 s (Kirischuk et al. 2007). In the cerebellum, electrical stimulation of parallel fibres induces local $[Na^+]_i$ responses in Bergmann glia, whereas activation of climbing

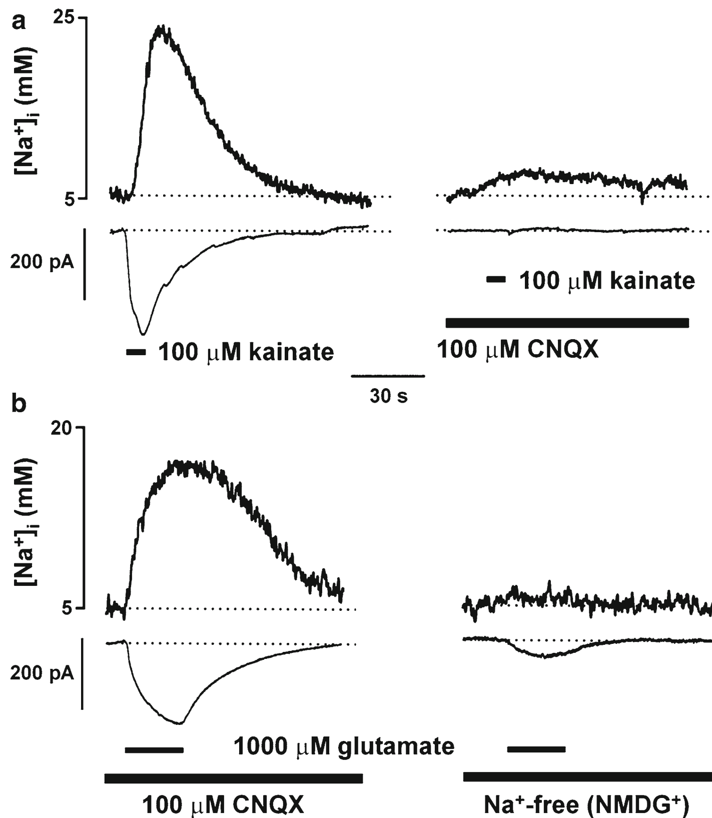

Fig. 25.1 *Glutamatergic stimulation triggers $[Na^+]_i$ elevation in Bergmann glial cells in situ in cerebellar slice.* (**a**) Simultaneous recordings of glutamate-induced inward current and $[Na^+]_i$ in response to cell stimulation with 100 µM of kainate, which opens AMPA receptors without triggering their desensitisation and is inactive against glutamate transporters (*left*). Both kainate-induced current and $[Na^+]_i$ transient are blocked by specific antagonist cyano-7-nitroquinoxaline-2,3-dione (CNQX, 100 µM) (*right*). (**b**) Similar to (a) inward currents and $[Na^+]_i$ were measured in Bergmann glial cells stimulated with 1 mM glutamate in the presence of 100 µM CNQX (the latter was added to exclude activation of AMPA ionotropic receptors) (*left*). Replacement of extracellular Na^+ by the organic cation N-methyl-D-glucamine ($NMDG^+$) eliminates both membrane current and $[Na^+]_i$ transient (*right*) (Modified from Kirischuk et al. (2007))

fibres activation triggers global $[Na^+]_i$ rise (Bennay et al. 2008). Thus, synaptic activity-induced intra-glial Na^+ responses show dependency on the synaptic input and significantly outlast the duration of synaptic activity.

25.7 Functional Significance of $[Na^+]_i$ Signalling

Rapid fluctuations of cytosolic Na^+ concentration can regulate numerous astroglial processes, which in turn can provide for local neuronal-glial communication (Fig. 25.2). In particular, elevation of $[Na^+]_i$ is directly coupled with generation of local $[Ca^{2+}]_i$ signals through favouring the reverse mode of NCX; indeed, $[Na^+]_i$ rises were directly demonstrated to induce additional Ca^{2+} influx that contributed to neurotransmitter-evoked $[Ca^{2+}]_i$ transients (Kirischuk et al. 1997). Our own data (Reyes et al. 2011) indicate that in cultured astrocytes, the reversal potential of NCX lies very close to the levels of resting membrane potential, and therefore, even moderate increases in $[Na^+]_i$ may rapidly lead to the NCX reverse operation. The NCX-mediated Ca^{2+} entry can in

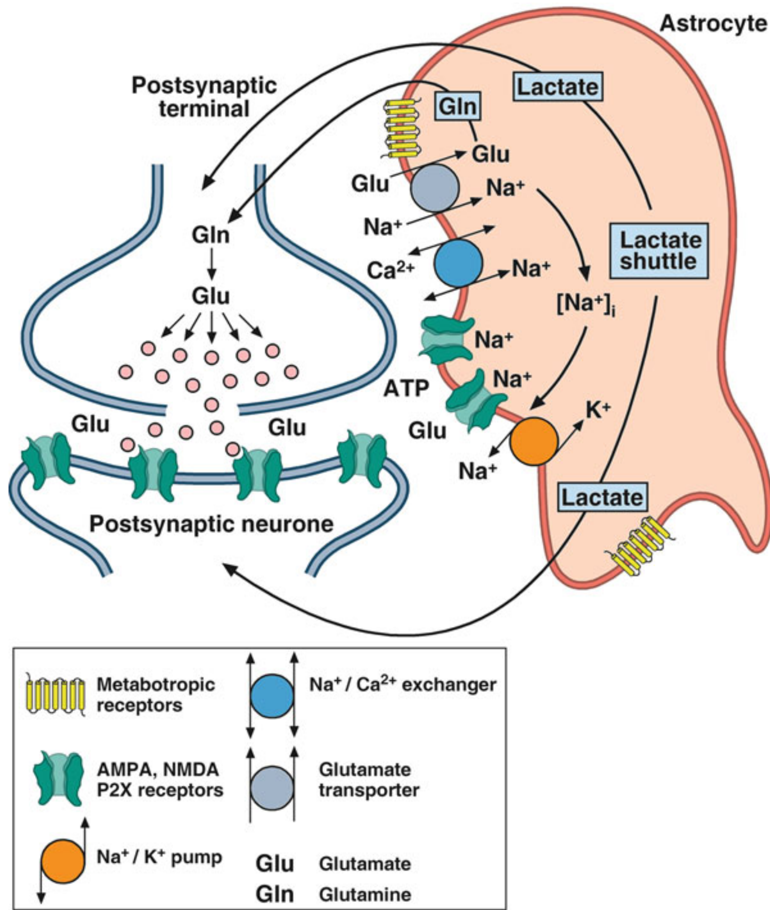

Fig. 25.2 *Possible physiological roles for* $[Na^+]_i$ *signalling. Local signalling mediated by ionotropic receptors and transporters in astroglial perisynaptic processes.* Synaptic release of neurotransmitters (glutamate and/or ATP) activates ionotropic receptors and glutamate transporters, which generate Na^+ influx and $[Na^+]_i$ elevation. Increases in $[Na^+]_i$ can assume a signalling role through modulating neurotransmitter transporters, switching the reverse mode of NCX and stimulating Na^+/K^+ pumps. This in turn can affect synaptic transmission and plasticity by modulating the time kinetic of glutamate removal from the cleft, through stimulating local metabolic support via lactate shuttle and through increase in extracellular K^+ buffering by the Na^+/K^+ pump

turn trigger exocytotic release of neurotransmitters from astroglia as have been demonstrated in several experimental paradigms (Benz et al. 2004; Paluzzi et al. 2007; Reyes et al. 2011).

Intracellular Na^+ is coupled to several other homeostatic systems. In particular, changes in $[Na^+]_i$ directly modulate $H^+/OH^-/HCO_3^-$ transport systems, which are fundamental for pH homeostasis, both intra- and extracellular (Deitmer and Rose 2010). Further, $[Na^+]_i$ directly controls the uptake of glutamate and GABA. Increases in $[Na^+]_i$ can significantly slow down or even reverse glutamate and/or GABA transporters. In fact, GABA transporter, because of its stoichiometry, is the most susceptible to regulation by $[Na^+]_i$, and even moderate rises in cytosolic Na^+ concentration can trigger GABA release through the reversed transporter (Heja et al. 2009; Wu et al. 2007). In addition, changes in $[Na^+]_i$ affect the activity of glutamine synthetase that further influences glutamate homeostasis (Benjamin 1987).

The second important target of $[Na^+]_i$ is the Na^+/K^+-ATPase, which has been found to

co-localise with NCX in cortical astrocytes at plasma membrane-endoplasmic reticulum junctions in the perisynaptic processes (Blaustein et al. 2002; Juhaszova and Blaustein 1997). The Na^+/K^+-ATPase in turn plays a critical role in regulation of neuronal-glial lactate shuttle (Magistretti 2006, 2009). In this scenario, local $[Na^+]_i$ increases will stimulate local supply of active synapses with energy substrate. In addition, $[Na^+]_i$ rise stimulates glucose uptake at the endfeet level thus supporting the lactate shuttle (Voutsinos-Porche et al. 2003).

25.8 Conclusions

Rapid fluctuations in cytosolic Na^+ in the astroglial processes, controlled by concerted activity of plasmalemmal Na^+ permeable receptors and channels together with Na^+ transporters and exchangers, may represent an additional layer of complexity in intracellular signalling, complementing more studied Ca^{2+} signalling and contributing to local bidirectional communication between a single synapse and perisynaptic glial processes.

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