Review

Sodium dynamics: another key to astroglial excitability?

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Astroglial excitability is largely mediated by fluctuations in intracellular ion concentrations. In addition to generally acknowledged Ca^{2+} excitability of astroglia, recent studies have demonstrated that neuronal activity triggers transient increases in the cytosolic Na^+ concentration ($[Na^+]_i$) in perisynaptic astrocytes. These $[Na^+]_i$ transients are controlled by multiple Na^+-permeable channels and Na^+-dependent transporters; spatiotemporally organized $[Na^+]_i$ dynamics in turn regulate diverse astroglial homeostatic responses such as metabolic/signaling utilization of lactate and glutamate, transmembrane transport of neurotransmitters and K^+ buffering. In particular, near-membrane $[Na^+]_i$ transients determine the rate and the direction of the transmembrane transport of GABA and Ca^{2+}. We discuss here the role of Na^+ in the regulation of various systems that mediate fast bidirectional communication between neurones and glia at the single synapse level.

Introduction

The mechanisms of astroglial excitability began to be considered about two decades ago when the general perception of neuroglia as passive connective tissue of the nervous system was challenged by the discoveries of electrical and Ca^{2+} responses of glial cells to neurotransmitters [1–3]. The general absence of electrical excitability of astrocytes (which are unable to generate action potentials) led to a concept of glial Ca^{2+} excitability based on cytoplasmic Ca^{2+} signals mainly originating from the endoplasmic reticulum (ER) [4]; this is a widespread mechanism for the majority of electrically non-excitable cells. Indeed, astrocytes ubiquitously express a multitude of metabotropic receptors coupled to inositol 1,4,5,-trisphosphate ($InsP_3$) metabolism and $InsP_3$-induced $[Ca^{2+}]_i$ signaling [5]. The seminal observation of Ann Cornell-Bell and colleagues [6], who demonstrated that Ca^{2+} waves can propagate through an astroglial syncytium, suggested that Ca^{2+} signals can be involved in long-range signaling by utilizing mechanisms very much distinct from electrically propagating action potentials. This finding was followed by experiments demonstrating that neuronal activity can trigger astroglial Ca^{2+} signals and astroglial Ca^{2+} waves both *in vitro* and *in vivo* [7–9]. Subsequent identification [10] ([11] for recent review) of astroglial Ca^{2+}-dependent exocytotic release of neurotransmitters added the missing link of Ca^{2+}-dependent astroglia-neuronal feedback to the idea of glial Ca^{2+} excitability.

By contrast, recent studies using various genetic manipulations to alter glial ER/Ca^{2+} signaling did not observe any effects on synaptic transmission and/or synaptic plasticity [12,13]. Such studies, albeit far from conclusive, have stirred much debate and controversy around the idea of astroglial excitability. Consequently, it is tempting to speculate that, in addition to Ca^{2+} release from the ER, astrocytes may possess alternative and/or additional signaling pathways. In particular, fluxes of sodium ions at the plasma membrane and dynamic fluctuations of cytoplasmic Na^+ concentration are appealing because they are: (i) linked to several cationic (calcium, proton, potassium) and anionic (chloride, glutamate, lactate) species, and (ii) constitute an interface between membrane signaling and metabolic pathways.

The nature of astroglial–neuronal complexes (generally referred to as glial–neuronal vascular units) calls for signaling systems that would, in a real time, coordinate synaptic activity with many homeostatic reactions of astrocytes that are fundamental for synaptic function. This could be perhaps accomplished by coordinated Ca^{2+} and Na^+ cytosolic dynamics, albeit other ionic species could also contribute. A single protoplasmic astrocyte covers some tens of thousands of synapses in rodents and up to 2 millions of synapses in humans by its perisynaptic processes (PAP), creating a 'tripartite synapse'/'synaptic cradle' [14]. Hence, PAPs can isolate synaptic inputs, thus conferring spatial specificity and controlling local ion, neurotransmitter and metabolic homeostasis which, to a great extent, define synaptic transmission [14].

We provide here an overview of recent studies which have addressed the role of intracellular Na^+ dynamics in astrocytic functions. We also discuss how astrocytes regulate

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$[Na^+]_i$, how astroglial $[Na^+]_i$ transients can be rapidly generated in response to neuronal activity, and how these $[Na^+]_i$ fluctuations influence a multitude of molecular cascades directly concerned with regulation of local homeostasis at the single synapse level.

Molecular physiology of sodium homeostasis in astroglia

Sodium ions cross astroglial membranes through channels, exchangers and transporters (Box 1). Voltage-gated Na^+ channels have been found in astrocytes *in vitro* and in acute brain and spinal cord slice preparations ([15] for review). However, their low density and the general reluctance of astrocytes to depolarize makes the voltage-gated Na^+ channel contribution to physiological Na^+ influx somewhat minor [16,17]. Most astrocytes express neurotransmitter receptors, which in physiological context mainly generate Na^+ currents [18]. Calcium permeability of astroglial neurotransmitter receptors is relatively low. For example, P_{Ca}/P_{Na} for GluA2-deficient AMPA receptors (AMPARs) is ~1, for glial NMDA receptors (NMDARs) ~3, and for purinergic $P2X_{1/5}$ receptors ~2 [19]. For $P2X_7$Rs, Ca^{2+} permeability depends on the pore formation and varies over a wide range. This makes Na^+ the main permeable cation flowing through open ligand-gated channels. Some astrocytes (for example those located in subfornical organ) express specific sodium channels activated by extracellular Na^+ concentration (Na_x channels) [20]. Finally, there are also some indications that astroglial cells may express functional members of the epithelial sodium channel (ENaC)/degenerin family [21], proton-activated acid-sensing ion channels (ASICs) [22], and several types of transient receptor potential (TRP) channels [23], which all can generate substantial Na^+ influx under physiological conditions.

The main astroglial plasma-membrane Na^+ transporter is represented by Na^+/K^+ ATPase; the $\alpha1/\alpha2$ subunit-containing pumps seem to be predominant forms. Sodium ions are also transported through Na^+/HCO_3^- cotransporters (NBC), Na^+/H^+ exchangers (NHE) and $Na^+/K^+/Cl^-$ cotransporter 1 (NKCC1), which are all present in the astrocyte plasmalemma (Box 1). Astrocytes ubiquitously express all three isoforms (NCX1, NCX2 and NCX3) of plasmalemmal Na^+/Ca^{2+} exchanger (Box 1, Figure I). According to the thermodynamics (stoichiometry of exchanges being $3Na^+:1Ca^{2+}$) 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 switch between forward/reverse operational modes is controlled by Na^+ and Ca^{2+} transmembrane ion gradients and the level of membrane potential. Depolarization and $[Na^+]_i$ increase favors reverse mode of NCX, whereas increases in $[Ca^{2+}]_i$ push the exchanger to the forward mode of operation. In astrocytes the NCX reversal potential (E_{NCX}) is close to the resting membrane potential (V_m approximately –80 mV). The NCX therefore dynamically fluctuates between forward/reverse modes and participates in both Ca^{2+} entry (observed in Bergmann glia [24] and cultured astrocytes [25–27]) and Ca^{2+} clearance [24]. In cultured cortical astrocytes NCX operates in reverse mode, even at the resting conditions [26]. Incidentally, the K^+-dependent versions of NCX (the SLC24 NCKX exchangers) were not detected in astrocytes, being most probably present exclusively in neurones [28].

Membrane Na^+ transport in astrocytes is also mediated by two families of Na^+-dependent neurotransmitter transporters: the excitatory amino acid (mainly glutamate) transporters 1 and 2 (EAAT1 and EAAT2) and GABA transporters of the GAT-1,3 subtypes (Box 1, Figure I). Transport of a single glutamate molecule (which at physiological pH is a monovalent anion) requires influx of three Na^+ ions, and efflux of one K^+ ion, down their respective concentration gradients; in addition glutamate brings one H^+ into the cell [29]. The net influx of cations manifests the electrogenic effect of glutamate transporter, which appears in a form of an inward current [30]. GATs mediate the symport of one uncharged GABA molecule, two Na^+ ions and one Cl^- ion [31]. Similarly to EAATs, GABA uptake via GATs is an electrogenic process, which generates an inward current.

Importantly, there are indications that neurotransmitter receptors (e.g., NMDARs), NCXs, Na^+/K^+ ATPases and EAATs/GATs are concentrated and colocalized in astroglial perisynaptic processes [32–34], which may indicate their strategic placement for local signaling.

Cytosolic sodium dynamics in astrocytes

Stimulation of astrocytes by neurotransmitters triggers large spatiotemporally organized transient $[Na^+]_i$ increases. For example, challenging astrocytes *in vitro* and *in situ* with ionotropic glutamate receptor agonists increases $[Na^+]_i$ by 10–25 mM [16,17,24,30,35,36]. When ionotropic receptors were pharmacologically blocked, exposure of astroglial cells to glutamate increased $[Na^+]_i$ by 10–20 mM; in this case the main Na^+ influx pathway was associated with activation of EAATs, as revealed by direct transporter current recordings (Figure 1a; [30]). Similarly, exposure to GABA triggers increases in $[Na^+]_i$ (with amplitudes up to 7 mM) in astrocytes in cortical slices (Figure 1b) [37].

Large $[Na^+]_i$ transients also occur in Bergmann glial cells and hippocampal astrocytes in acute slices in response to electrical stimulation of neuronal afferents [24,30,38]. The $[Na^+]_i$ transients in response to stimulation of neuronal inputs were accompanied with glial synaptic currents mediated by both glutamate ionotropic receptors and transporters [30,38,39]. Even short (5–10 pulses) stimulation of neuronal afferents elevated $[Na^+]_i$ by 10–15 mM (Figure 1c) [30]. Glial $[Na^+]_i$ signals were found to be dependent on the synaptic input: in cerebellar Bergmann glial cells electrical stimulation of parallel fibers induced $[Na^+]_i$ transients in glial cell processes, whereas activation of climbing fibers triggered a rise in global $[Na^+]_i$ [38]. Focal initiation of astroglial $[Na^+]_i$ rise triggered propagating $[Na^+]_i$ waves that spread through glial syncytia both in culture (Figure 2a) [40] and in hippocampal slices (Figure 2b) [41]. These glial $[Na^+]_i$ waves were dependent on connexin channels and were inhibited by appropriate pharmacological blockers or by genetic deletion of connexins 30 and 43 (Cx30/43) (Figure 2b) [41]. Failure in rapid redistribution of Na^+, K^+ and glutamate via astroglial networks resulted in astrocyte swelling and scaling-up of