TRP Channels Coordinate Ion Signalling in Astroglia

Alexei Verkhratsky, Reno C. Reyes, and Vladimir Parpura

Abstract Astroglial excitability is based on highly spatio-temporally coordinated fluctuations of intracellular ion concentrations, among which changes in Ca^{2+} and Na^{+} take the leading role. Intracellular signals mediated by Ca^{2+} and Na^{+} target numerous molecular cascades that control gene expression, energy production and numerous homeostatic functions of astrocytes. Initiation of Ca^{2+} and Na^{+} signals relies upon plasmalemmal and intracellular channels that allow fluxes of respective ions down their concentration gradients. Astrocytes express several types of TRP channels of which TRPA1 channels are linked to regulation of functional expression of GABA transporters, whereas TRPV4 channels are activated following osmotic challenges and are up-regulated in ischaemic conditions. Astrocytes also ubiquitously

A. Verkhratsky (✉)
Faculty of Life Sciences, The University of Manchester, Oxford Road, Manchester M13 9PT, UK

Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, Bilbao 48011, Spain
e-mail: Alexej.Verkhratsky@manchester.ac.uk

R.C. Reyes
Department of Neurobiology, Center for Glial Biology in Medicine, Atomic Force Microscopy & Nanotechnology Laboratories, Civitan International Research Center, Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, 1719 6th Avenue South, CIRC 429, Birmingham, AL 35294, USA

Department of Psychiatry, Langley Porter Psychiatric Institute, University of California San Francisco, 401 Parnassus Avenue, San Francisco, CA 94143, USA

V. Parpura (✉)
Department of Neurobiology, Center for Glial Biology in Medicine, Atomic Force Microscopy & Nanotechnology Laboratories, Civitan International Research Center, Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, 1719 6th Avenue South, CIRC 429, Birmingham, AL 35294, USA

Department of Biotechnology, University or Rijeka, Rijeka 51000, Croatia
e-mail: vlad@uab.edu; vparpura@biotech.uniri.hr

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express several isoforms of TRPC channels of which heteromers assembled from TRPC1, 4 and/or 5 subunits that likely act as stretch-activated channels and are linked to store-operated Ca^{2+} entry. The TRPC channels mediate large Na^{+} fluxes that are associated with the endoplasmic reticulum Ca^{2+} signalling machinery and hence coordinate Na^{+} and Ca^{2+} signalling in astroglia.

Keywords Astrocyte · Ca^{2+} signalling · Na^{+} signalling · Metabotropic receptors, endoplasmic reticulum · TRPC channels · TRPCA1 · TRPV4 · Store-operated Ca^{2+} entry · Stretch-activated channels · Mechanosensitivity · Volume regulation · Plasticity · Brain homeostasis

Contents

1 Astrocytes: The Homeostatic Cells of the CNS

Evolution of the central nervous system (CNS) progressed through specialisation of the cellular elements composing neural networks. Functional dissociation between executive cellular branch represented by electrically excitable neurones and housekeeping branch represented by electrically non-excitable glial cells developed immediately after appearance of centralised masses of neural cells and attained highest degree of complexity in the mammalian CNS (Kettenmann and Ransom 2013; Verkhratsky and Butt 2013). Astroglia refers to a highly heterogeneous cell population present in the grey matter and in the white matter of the brain and of the spinal cord that are responsible for a remarkable array of homeostatic functions that control the CNS environment and provide for seemingly slick operation of neural cells (Nedergaard and Verkhratsky 2012; Parpura and Verkhratsky 2012). Astrocytes, in particular, are fundamentally important for rapid regulation of extracellular ions (Kofuji and Newman 2004; Olsen and Sontheimer 2008) and neurotransmitters (Conti et al. 2004; Danbolt 2001), that, to a large extent, shape neuronal excitability and synaptic transmission. Astrocytes are critical for providing glutamatergic and GABA-ergic neurones with glutamine, which is indispensable for

maintaining releasable pool of these transmitters (Hertz et al. 1999); similarly, astrocytes are mainly responsible for adenosine turnover (Boison et al. 2010). Astrocytes, unlike neurones, can synthesise glutamate de novo owing to the entry of pyruvate to the citric acid cycle via astrocyte-specific mitochondrial enzyme pyruvate carboxylase (Hertz and Zielke 2004). Astroglial cells are also capable of releasing various neurotransmitters and neuromodulators that provide for regulation of synaptic connectivity and plasticity (Henneberger et al. 2010; Parpura and Zorec 2010). Astrocytes support neuronal energetics with lactate (Magistretti 2011) and hold at bay extracellular accumulation of reactive oxygen species using nonenzymatic antioxidant defences, such as ascorbate and glutathione (Fernandez-Fernandez et al. 2012; Swanson et al. 2004). Astroglial cells also contribute to regulation of brain microcirculation by linking neuronal activity with functional hyperaemia (Carmignoto and Gomez-Gonzalo 2010; Iadecola and Nedergaard 2007). Finally astroglial cells are fundamental elements of brain defence through evolutionary conserved multistage programmes of reactive astrogliosis (Sofroniew 2009). To maintain all these functions, astroglial cells are in need of real-time monitoring of their immediate environment, including neuronal activity, with rapid activation of multiple intracellular signalling cascades regulating varieties of molecules responsible for homeostatic response.

2 Ion Signalling Defines Astroglial Excitability

Astrocytes are electrically non-excitable cells incapable of producing plasmalemmal regenerative responses based on coordinated activity of voltage-gated ion channels, that is, action potentials that underlie signalling in neuronal networks. There are however numerous types of voltage-gated channels expressed in astroglia (Verkhratsky and Steinhauser 2000), although densities of Na^+ and Ca^{2+} permeable channels (otherwise necessary proviso for generation of action potentials) are low and membrane depolarisation is prevented by large K^+ permeability and shunting through gap junctions. Nonetheless astrocytes are mounting active responses to external stimulation (with chemical and mechanical stimulation being physiologically relevant) by producing changes in intracellular ion concentration coordinated in spatio-temporal domains.

Intracellular Ca^{2+} signals were the first kind of ionic signalling recognised to be universally present in astroglia. Early experiments have found that stimulation of cultured astrocytes with neurotransmitters (such as glutamate) or with mechanical displacement of membrane produced transient changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) that were able to propagate through a glial monolayer in the form of Ca^{2+} waves (Charles et al. 1991; Cornell Bell et al. 1990; Finkbeiner 1993). Subsequently, experiments in vitro demonstrated that astrocytes are capable of expressing numerous receptors linked to Ca^{2+} signalling (Verkhratsky and Kettenmann 1996). These receptors, triggering Ca^{2+} signals, were further characterised in situ revealing

remarkable region-dependent variability, with receptors' patterns matching immediate neurochemical environment, that is, nearby neurotransmission (Verkhratsky et al. 1998). In recent years, astroglial Ca^{2+} signals and astroglial Ca^{2+} waves were also identified in vivo and linked to various forms of sensory stimulation (Kuga et al. 2011; Wang et al. 2006). Importantly, astroglial Ca^{2+} signals can induce neuronal responses (Nedergaard 1994; Parpura et al. 1994), although the detailed physiological consequences of such interactions remained to be clarified that warrant further investigations (Gourine et al. 2010; Poskanzer and Yuste 2011).

The second kind of intracellular ion signalling in astroglia is associated with transient changes in cytosolic concentration of Na^+ ($[Na^+]_i$). It appears that physiological (i.e. chemical and mechanical) stimulation triggers rapid and substantial transient increases in $[Na^+]_i$ in astrocytes in culture (Reyes et al. 2012; Rose and Ransom 1996) and in situ (Kirischuk et al. 1997; Langer and Rose 2009). These $[Na^+]_i$ transients also follow synaptic stimulation (Kirischuk et al. 2007; Langer and Rose 2009), and Na^+ can propagate through astroglial syncytia in the form of Na^+ waves ((Langer et al. 2012; Rose and Ransom 1997), for detailed description of glial Na^+ signalling, see (Kirischuk et al. 2012; Rose and Karus 2013) and references therein). Importantly, these $[Na^+]_i$ fluctuations are involved in regulation of multiple astroglial homeostatic cascades (Kirischuk et al. 2012). The sources of Na^+ signalling in astroglia are associated with Na^+ influx through ion channels and Na^+ transport through multiple Na^+ secondary transporters. Of these the Na^+-dependent glutamate and GABA transporters are of particular importance, because they are activated in the course of synaptic transmission (Kirischuk et al. 2007; Unichenko et al. 2012). The above-mentioned two forms of ion (Na^+ and Ca^{2+}) signalling are interlinked through, for example, the plasmalemmal sodium-calcium exchangers (NCXs) and transient receptor potential (TRP) channels, the role of which will be discussed in detail below.

The functional consequences of Ca^{2+} and Na^+ signalling in astrocytes are many and they are mediated through multiple molecular cascades sensitive to cytosolic ion concentrations (see Table 1 for selected targets). Ions regulate molecular function either through selective binding (which is common for Ca^{2+} sensors) or through changes in electrochemical driving force across cellular membranes (which is more common for Na^+ targets). Another important determinant for ion signalling is focalisation, that is, microdomains of high ion concentrations that are spatially confined to the functionally relevant areas. Spatial restriction of Ca^{2+} signals are mainly achieved through cytosolic Ca^{2+} buffers that limit Ca^{2+} diffusion; mechanisms of localisation of Na^+ signals remain uncharacterised. Of note, however, the sites of Na^+ entry are often co-localised with Na^+ pumps and transporters; these latter can act as dynamic Na^+ buffers and contribute to focalisation of $[Na^+]_i$ fluctuations.

Table 1 Selected functional and molecular targets of Ca^{2+} and Na^{+} signals in astroglia

Functional responses	Molecular targets	References
Ca^{2+} signals		
Gene expression	Transcriptional factors/regulators (e.g. CREB/DREAM)	(Cebolla et al. 2008; Zhao and Brinton 2004)
Exocytosis	Synaptotagmins (functionally uncomfirmed)	(Mittelsteadt et al. 2009; Zhang et al. 2004)
	Calcineurin/calmodulin-mediated modulation of secretory machinery	(Reyes et al. 2011)
Mitochondrial ATP production	Ca^{2+}-sensitive mitochondrial dehydrogenases; pyruvate dehydrogenase phosphatase; F_1–F_0 ATP synthase	(Tarasov et al. 2012)
Ca^{2+} transport	Plasmalemmal Ca^{2+} ATPase (PMCA); sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA)	(Burdakov et al. 2005; Reyes et al. 2012)
Na^{+} signals		
K^{+} buffering	Inward rectifying K^{+} channel ($K_{ir}4.1$)	(Kucheryavykh et al. 2012)
	Na^{+}/K^{+} ATPase	(Walz and Hertz 1984)
	$Na^{+}/K^{+}/Cl^{-}$ co-transporter (NKCC1/SLC12A2)	(MacVicar et al. 2002)
Glutamate-glutamine shuttle:		
Glutamate uptake	Excitatory amino acid transporters 1, 2 (EAAT1/SLCA2, EAAT2/SLCA3)	(Anderson and Swanson 2000)
Glutamine transport	Na^{+}/H^{+}-dependent sodium-coupled neutral amino acid transporters (SN1/SNAT3/SLC38A3 and SN2/SNAT5/SLC38A5)	(Broer and Brookes 2001; Hertz 1979; Uwechue et al. 2012)
Glutamine-GABA shuttle: GABA uptake	GABA transporter (GAT3/SLC6A11)	(Unichenko et al. 2012)
Glycine uptake	Glycine transporter 1 (GlyT1/SLC6A9)	(Gomeza et al. 2003)
Plasmalemmal Na^{+}/Ca^{2+} exchange	Sodium calcium exchangers (NCX1/SLC8A1, NCX2/SLC8A2 and NCX3/SLC8A3)	(Kirischuk et al. 2007, 2012; Reyes et al. 2012)
Mitochondrial Na^{+}/Ca^{2+} exchange	Mitochondrial sodium calcium exchanger (NCLX/SLC8B1)	(Parnis et al. 2013; Reyes and Parpura 2008)
pH homeostasis: H^{+} transport	Na^{+}/H^{+} exchanger (NHE1/SLC9A1)	(Kintner et al. 2005)
pH homeostasis: HCO_3^{-} transport	Sodium bicarbonate co-transporter (NBC/SLC4A5)	(Deitmer and Rose 2010; Lascola and Kraig 1997)
Lactate shuttle	Na^{+}/K^{+} ATPase	(Magistretti 2011; Pellerin and Magistretti 1996, 2012)

3 Astroglial Na^+/Ca^{2+} Channels

Astrocytes, in physiological conditions, express several sets of cationic channels permeable to both Na^+ and Ca^{2+}. There is no firm evidence for expression of highly selective Ca^{2+} channels in astroglial cells in situ. There are indications for expression of several types of voltage-dependent Ca^{2+} channels in astrocytes in culture, which, however have not been confirmed for mature astrocytes neither in brain slices nor in vivo (reviewed in Parpura et al. (2011)). Voltage-gated Ca^{2+} channels can be confined to immature astroglial precursors, to NG-2 cells (with which astrocytes can be often mistaken) and to reactive astroglia (Parpura et al. 2011; Verkhratsky et al. 2012). Similarly, highly selective Ca^{2+}-release activated Ca^{2+} channels (of I_{CRAC} variety) have not been hitherto recorded from mature astroglial cells in brain tissue, while Orai channels and their respective currents have been recently recorded in primary cultured astrocytes and astroglial cell lines (Moreno et al. 2012; Motiani et al. 2013). Likewise, evidence for expression of voltage-gated Na^+ channels in cultured astroglia (Black et al. 2010) have not been corroborated by direct electrophysiological recordings in situ. It appears that the majority of ion channels expressed in astroglial membrane is permeable to both Na^+ and Ca^{2+} supporting the idea of interwoven intracellular Na^+ and Ca^{2+} excitability of astroglia.

Astrocytes express several types of cationic ionotropic receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and N-methyl-D-aspartate (NMDA) receptors and purinergic P2X receptors (Lalo et al. 2006, 2008; Steinhauser and Gallo 1996). These receptors have (in contrast to neurones) an intermediate to small Ca^{2+} permeability (Pankratov et al. 2009). In some types of astroglia, the AMPA receptors lack the GluA2 subunit which underlies their Ca^{2+} permeability ($P_{Ca}/P_{monovalent} \sim 1$ (Burnashev et al. 1992; Muller et al. 1992)). This, however, corresponds to ~4 % of fractional Ca^{2+} current, which together with rapid physiological AMPA receptor desensitisation very much limits Ca^{2+} entry. In Bergmann glial cells, Ca^{2+} permeable AMPA receptors have minimal, if any, contribution to Ca^{2+} signals (Kirischuk et al. 1999). Similarly, astroglial NMDA and $P2X_{1/5}$ receptors have relatively low Ca^{2+} permeability ($P_{Ca}/P_{monovalent} \sim 3$ and $P_{Ca}/P_{monovalent} \sim 2$, respectively (Palygin et al. 2010)). There is fragmentary evidence (Sharma and Vijayaraghavan 2001) for astroglial expression of α7 Ca^{2+} permeable nicotinic cholinoreceptors (α7nAChRs), although another study in hippocampal slices produced somewhat inconclusive results (Shen and Yakel 2012), and specific parameters and functional role of astroglial α7nAChRs similarly remain unknown. Another important pathway for membrane Na^+ entry in astroglia is represented by TRP channels.

4 TRP Channels as Multi-ion Carriers

The TRP channel family (for the somewhat controversial history of its discovery, see (Hardie 2011; Minke 2010; Montell 2011)) is widely present in many cell types of all multicellular organisms, from *Caenorhabditis elegans* to mammals, although the phylogenetic roots of this channel are found in yeasts (the TRPY channel family (Venkatachalam and Montell 2007)). There are 28 members of the superfamily in vertebrates, of which 27 are present in humans (Nilius et al. 2012; Owsianik et al. 2006; Pedersen et al. 2005) and classified into 6 subfamilies. The TRP channels are fundamental for all types of sensing including, thermal sensation, nociception, chemoception, equilibrioception and interoception (Nilius and Appendino 2013; Nilius and Owsianik 2011; Vennekens et al. 2012). The TRP channels are cationic channels permeable to multiple cations with great heterogeneity of permeation properties (Owsianik et al. 2006). They are found in the CNS, being expressed in cells from all regions of the brain and the spinal cord with particularly high expression of TRPV, TRPC and TRPM channels, and more restricted expression of TRPA1, TRPP1 and TRP-ML proteins (for many details and exhaustive reference list, see (Nilius 2012; Vennekens et al. 2012)).

5 TRP Channels in Astroglia

5.1 TRPA1 Channels

TRPA1 (where 'A' stands for ankyrin) is the only member of this subfamily identified in mammals (Nilius et al. 2011) with high single channel conductance (~110 pS) and relatively high Ca^{2+} permeability ($P_{Ca}/P_{monovalent}$ ~ 5.9). This Ca^{2+} permeability can be increased even further upon channel activation that is accompanied with pore dilation. In dilated state the $P_{Ca}/P_{monovalent}$ is ~7.9, corresponding to fractional Ca^{2+} current of ~23 % (Nilius et al. 2011). These TRPA1 channels can be activated by noxious cold (below 17 °C), by pungent substances derived from plants, by growth factors (via G-protein-coupled receptors) and by pro-inflammatory factors (Nilius et al. 2012).

Functional expression of TRPA1 channels was suggested for hippocampal astrocytes, although neither specific mRNA nor TRPA protein was detected in these cells (Shigetomi et al. 2012). Nonetheless a complex of Ca^{2+} imaging (with a genetically encoded Ca^{2+} probe Lck-GCaMP that monitors near-membrane $[Ca^{2+}]$), electrophysiology, silencing RNA and pharmacology provided reasonably convincing evidence for operation of these channels in sub-population of astroglia (Shigetomi et al. 2012). The fundamental observation was a detection (in cultured astrocytes) of near-membrane local spontaneous $[Ca^{2+}]_i$ transients (called by the authors 'spotty' Ca^{2+} signals) that were inhibited by Gd^{3+} and La^{3+} as well as by broad spectrum TRP channel antagonist HC 030031. Similarly these 'spotty' Ca^{2+}

signals were blocked by anti-TRP silencing RNA, whereas the TRPA1 agonist allyl isothiocyanate (AITC) increased frequency of these events; AITC also activated currents in voltage-clamped astrocytes. Further studies have found evidence for functional activity of TRPA1 channels in astroglial cells in situ in hippocampal slices. Activity of TRPA1 channels apparently contributed to setting the resting $[Ca^{2+}]_i$ in astrocytes (both in cultures and in situ) and inhibition of these channels resulted in a significant (from ~120 to ~50 nM) decrease in basal $[Ca^{2+}]_i$. This decrease in resting $[Ca^{2+}]_i$ in turn reduced functional expression of astroglial GABA plasmalemmal GAT-3 transporters, which, as authors suggested, resulted in an elevated extracellular concentration of GABA, desensitization of $GABA_A$ receptors in neighbouring hippocampal neurones and hence a decrease in the inhibitory synaptic transmission (Shigetomi et al. 2012).

5.2 TRPC Channels

Mammalian TRPC ('C' denotes canonical) channels are represented by seven members (TRPC1–7) which are all cationic channels with $P_{Ca}/P_{monovalent}$ varying between 1 and 9 (Owsianik et al. 2006). These channels can be activated by phospholipase C, by diacylglycerol (DAG) and by mechanical stimulation, and are responsible for store-operated Ca^{2+} entry in some types of cells. The TRPC channels can form both homo- and heteromeric channels, which underlie substantial heterogeneity in their biophysical properties (Nilius et al. 2007).

Embryonic cultured astrocytes (also often referred to as astrocytes type I) express mRNA for TRPC1 to TRPC6 (Grimaldi et al. 2003; Pizzo et al. 2001) and were reported to produce Ca^{2+} fluxes and $[Ca^{2+}]_i$ oscillations in response to oleyl-acetyl-glycerol (an analogue of DAG) and following stimulation of glutamate receptors and endoplasmic reticulum (ER) store depletion. In spinal astrocytes, the mRNAs for TRPC1, 2, 3, 4 and 6 were detected (Miyano et al. 2010). At the protein level relatively high expression of TRPC1 channel was detected in the embryonic astroglial cultures. It appeared that TRPC1 channels were located in the portions of plasmalemma closely associated with the ER (i.e. at plasmalemma-ER junctions) and, moreover, TRPC1 proteins were co-immunoprecipitated with inostiol 1,4,5 trisphospate ($InsP_3$) receptors and ER Ca^{2+}-ATPases of SERCA 2b subtype suggesting intimate functional relations between ER receptors, Ca^{2+} transporters and plasmalemmal TRPC1-containig channels (Golovina 2005). Likewise, co-immunoprecipitation of TRPC1 channels, $InsP_3$ receptors type II and Homer proteins was found in cortical astrocytes cultured from 3- to 5-day-old rats (Weerth et al. 2007). Similar co-localisation of TRPC4 channels with ZO-1 scaffolding proteins was detected in cultured foetal human astrocytes (Song et al. 2005). Besides TRPC1 expression, TRPC4, TRPC5 and TRPC6 proteins were also detected in cultured and freshly isolated embryonic astrocytes (Beskina et al. 2007).

In primary astrocytes cultured from visual cortices of newborn rats or freshly isolated from the same region of 1-, 8- and 55-day-old rats, expression of TRPC1,

TRPC4 and TRPC5 channels was detected in Western blots and their cellular localisation was mapped with immune labelling showing that TRPC1 channels were predominantly localised to the plasma membrane (Malarkey et al. 2008). The percentage of astrocytes expressing TRPC isoforms increased with age. At 1 day of age, percentage of astrocytes expressing TRPCs was 47 %, 7 % and 70 % for TRPC1, TRPC4 and TRPC5 proteins, respectively, whereas at 55 days of age all astrocytes expressed all three isoforms (Malarkey et al. 2008). Indeed, several TRPC isoforms are expressed in the brain, where the predominant types are represented by TRPC1, 4 and 5 subunits that are generally believed to form heteromers, in which TRPC1 acts as an obligatory, channel forming subunit and TRPC4/5 function as ancillary ones (Hofmann et al. 2002; Strubing et al. 2001).

Activation of TRPC1 channels in astroglia has been observed in various physiological and pathophysiological contexts. The TRPC1 channels contribute to $[Ca^{2+}]_i$ transients induced by stimulation of purinergic and glutamatergic metabotropic receptors (Malarkey et al. 2008), because treatment of astrocytes with anti-TRPC blocking antibody substantially reduced the plateau phase, as well as a component of the peak, of these Ca^{2+} responses (Fig. 1a). The TRPC1 channels are also instrumental for astroglial Ca^{2+} signalling following mechanical stimulation. The ability of TRPC1 to act as a stretch-activated polycationic channel (first identified as Na^+, K^+, Ca^{2+} and Mg^{2+} mechanosensitive cation channel MscCa) was initially demonstrated in frog oocytes, and the similarity between MscCa and TRPC1 was confirmed following heterologous expression studies (Maroto et al. 2005). Of note, the role of TRPC channels as mechanosensors remains controversial, while recent discoveries of Piezo1/2 channels open new avenues in understanding mechanisms of mechano-transduction (Nilius and Honore 2012). Be it all as it may, it was demonstrated that inhibition of TRPC1 channels substantially reduces $[Ca^{2+}]_i$ transients induced by mechanical stimulation of cultured astrocytes (Malarkey et al. 2008; Reyes et al. 2013) (Fig. 1b) as well as consequential Ca^{2+}-dependent glutamate release from these glial cells (Fig. 1c). The TRPC channels in cortical astrocytes are also activated by hypo-osmotic shock, and the resulting $[Ca^{2+}]_i$ elevation triggers translocation of aquaporin-1 water channels to the plasma membrane that increases water transport (Conner et al. 2012). The TRPC6 channels were claimed to contribute to Ca^{2+} entry following stimulation of interleukin-1β (IL-1β) receptors in embryonic astrocytes (Beskina et al. 2007).

5.3 *TRPC Channels as Molecular Substrate of Store-Operated Ca^{2+} Entry in Astroglia*

The store-operated (also known as 'capacitative') Ca^{2+} entry (SOCE) mechanism (Putney 1990, 2007) is expressed in virtually all types of non-excitable cells and in some excitable cells. This mechanism is operated by a dynamic molecular link between the ER and the PM. The molecular sensor that monitors the intra-ER

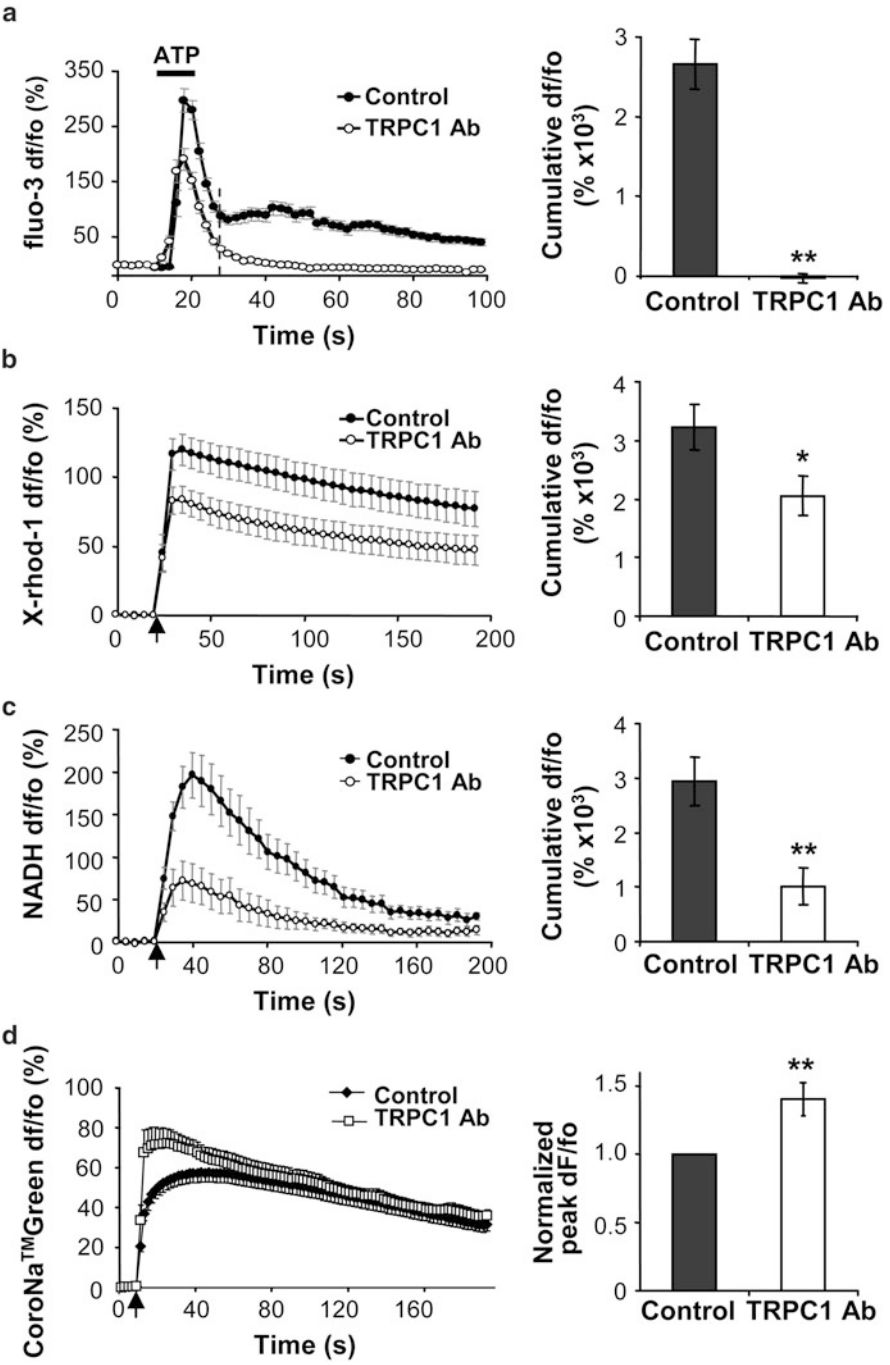

Fig. 1 The role of TRPC1 in intracellular Ca^{2+} and Na^{+} dynamics in cultured astroglia. (**a**) TRPC1 plays a role in receptor activation-elicited intracellular Ca^{2+} elevations in astrocytes. Application of ATP (100 μM) to astrocytes from rat visual cortex results in a biphasic intracellular Ca^{2+} response:

Ca^{2+} concentration is represented by the stromal interacting molecule proteins (STIM1 and STIM2). Upon ER Ca^{2+} depletion, STIM molecules oligomerise and drift towards the ER-PM junction where they interact with and activate plasmalemmal Ca^{2+} channels. These latter are (i) I_{CRAC} channels formed by Orai proteins and/or (ii) TRPC channels (for review of and references about molecular physiology of SOCE, see (Cahalan 2009; Carrasco and Meyer 2011; Feske et al. 2006; Owsianik et al. 2006; Parekh 2010; Parekh and Penner 1997; Soboloff et al. 2012; Zeng et al. 2008)). Channels formed by Orai and TRPC have distinct biophysical identity and their corresponding currents and functional responses can be easily distinguished.

This SOCE pathway is functioning in virtually all types of neuroglial cells (Hartmann and Verkhratsky 1998; Malarkey et al. 2008; Moller et al. 1997; Muller et al. 2013; Paez et al. 2009; Pivneva et al. 2008; Pizzo et al. 2001; Reyes and Parpura 2009; Toescu et al. 1998; Tuschick et al. 1997). To the best of our knowledge, characteristic I_{CRAC} channels have not been hitherto recorded from mature astrocytes and evidence about functional operation of Orai/STIM complex derives from neoplastic cell lines and astrocytes in vitro (Moreno et al. 2012; Motiani et al. 2013).

The role for TPRC1 channels in SOCE in astroglial cells is based on functional studies deploying immunological inhibition and down-regulation of TRPC1 channels' expression in combination with Ca^{2+} imaging. The antisense RNA knock-down of TRPC1 as well as inhibition of the channel with blocking antibody directed at an epitope in the pore forming region of the TRPC1 protein substantially reduced SOCE (activated either following metabotropic stimulation or following ER store depletion with SERCA blockers, namely, cyclopiazonic acid or thapsigargin) in cultured cortical astrocytes (Golovina 2005; Malarkey et al. 2008). As alluded to earlier, this TRPC1 inhibition underlies reduction of plateau phase of $[Ca^{2+}]_i$ transients induced by ATP in astrocytes in vitro (Golovina 2005; Malarkey et al. 2008). Similarly the SOCE-mediated plateau of Ca^{2+} responses

Fig. 1 (continued) the initial transient Ca^{2+} elevation and sustained (plateau) Ca^{2+} elevation. Intracellular Ca^{2+} measurements were obtained using the Ca^{2+} indicator fluo-3. If TRPC1 containing channels are blocked by incubating cells with an antibody against TRPC1, the sustained (plateau) Ca^{2+} elevation, reporting on SOCE, is abolished. *Vertical dashed line* indicates the initial point of a sustained plateau Ca^{2+} response, of which cumulative is shown in bar graph. (**b**, **c**) TRPC1 plays a role in mechanically elicited intracellular Ca^{2+} responses in astrocytes and resulting Ca^{2+}-dependent glutamate release from these glial cells. (**b**) Mechanical stimulation causes cytoplasmic Ca^{2+} elevations in astrocytes, as recorded using the Ca^{2+} indicator X-rhod-1. (**c**) Glutamate release from astrocytes, reported by an increase in extracellular NADH fluorescence, can be induced by mechanical stimulation. Both responses (Ca^{2+} and glutamate) are reduced when astrocytes were incubated with TRPC1 antibody. (**d**) TRPC1 plays a role in mechanically elicited intracellular Na^+ responses in astrocytes. Mechanical stimulation causes cytoplasmic Na^+ elevations in astrocytes, as recorded using the Na^+ indicator CoroNa^{TM}Green. The peak Na^+ responses are enhanced when astrocytes were incubated with TRPC1 antibody. *Point* and *bars* indicate means ± SEMs. *Asterisks* indicate a significant change of measurements compared with the control group ($^{*}p < 0.05$, $^{**}p < 0.01$). *Arrows* in b–d indicate the time of mechanical stimulation. (**a**–**c**: Modified from Malarkey et al. (2008); **d**: Modified from Reyes et al. (2013))

to glutamate, ATP and endothelin-1 were inhibited by Zn^{2+}, Gd^{3+} and La^{3+} in astrocytes in culture and in hippocampal slices (Kresse et al. 2005). This inhibition likely reflects upon the action of these metal ions on the TRPC1 channel. In-depth analysis of the SOCE induced by activation of bradykinin receptors in cultured astrocytes isolated from the cortex of newborn mice revealed the leading role of TRPC1 and to a lesser extent TRPC3 isoforms (Akita and Okada 2011). In contrast, in spinal astrocytes (stimulated by neurokinin-1 receptor agonists substance P and GR73632) the SOCE was predominantly mediated by TRPC3 channels being sensitive to specific inhibitor ethyl-1-(4-(2,3,3-trichloroacrylamide)phenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate or Pyr3 (Miyano et al. 2010). Incidentally, chronic (3–21 days) treatment of cultured astrocytes with the serotonin $5\text{-}HT_{2B}$ receptor agonist fluoxetine substantially (by 50–90 %) reduced TRPC1-dependet SOCE (Li et al. 2011). The expression of TRPC1 and TRPC1-mediated SOCE in astrocytes also seems to be mediated by amyloid precursor protein (APP) and knocking out of APP substantially reduced both (Linde et al. 2011).

All in all, the wealth of data seems to indicate that TRPC1 (most likely heteromeric with TRPC3, 4 and 5) is, to a large extent, responsible for astroglial SOCE. This is, incidentally, contrasting to microglia, where SOCE is mostly (if not exclusively) mediated by Orai-composed I_{CRAC} channels (Verkhratsky and Parpura 2013).

5.4 TRPV Channels

TRPV ('V' for vanilloid) channels family covers six members which are activated by various chemical, thermal, and noxious stimuli; TRPV4 channels are also sensitive to osmotic pressure. All TRPV channels are Ca^{2+} permeable with $P_{Ca}/P_{monovalent}$ between 1 and 10 for TRPV1–4, and $P_{Ca}/P_{monovalent} > 100$ for TRPV5 and 6 (Owsianik et al. 2006).

Astrocytes in cortex and in hippocampus express TRPV4 channels (localised mainly in their processes) that are involved in osmotic sensing and together with aquaporins (AQP) contribute to cell volume regulation (Bai and Lipski 2010; Benfenati et al. 2007; Butenko et al. 2012; Liu et al. 2006). These TRPV4 channels were found to be activated by hypotonicity that triggered substantial Ca^{2+} influx resulting in $[Ca^{2+}]_i$ elevation. This could be blocked by the TRPV inhibitor ruthenium red (Benfenati et al. 2007). The TRPV4-mediated outwardly rectifying currents were also monitored in voltage-clamp configuration following stimulation with the selective TRPV4 agonist 4-α-phorbol 12,13-didecanoate (Benfenati et al. 2007). Similarly, TRPV-4-mediated currents and $[Ca^{2+}]_i$ transients were recorded from astrocytes in hippocampal slices. Both events were blocked by ruthenium red and the TRPV-4 selective inhibitor RN1734 (Butenko et al. 2012). In cortical astroglia, TRPV4 were shown to interact with AQP-4; the resulting TRPV4-AQP4 complexes were critical for regulatory volume decrease ensuing hypo-osmotic shock (Benfenati et al. 2011; Benfenati and Ferroni 2010).

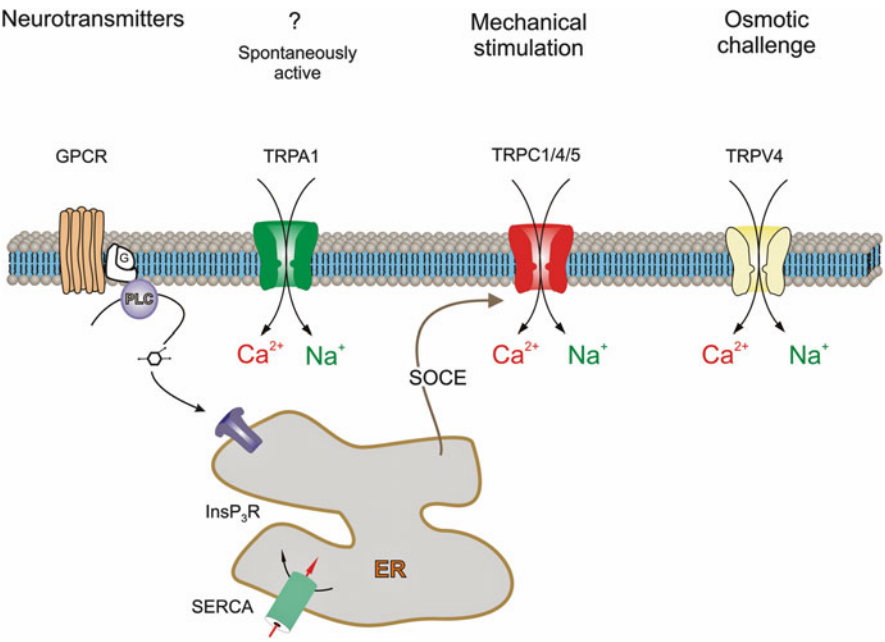

Fig. 2 Variety of astroglial TRP channels. Note the link between metabotropic stimulation and TRPC channels through the ER and store-operated Ca^{2+} entry

Recently (Mannari et al. 2013), TRPV1 channels were also detected (by PCR, Western blotting and immunohistochemistry) in astrocytes in circumventricular organs (more specifically within the organum vasculosum of the lamina terminalis, subfornical organ and area postrema) that contain chemosensitive area of the brain. These channels were found especially abundant in the thick processes of astrocytes that surround blood vessels, and hence could presumably be activated by a blood-borne stimulus. In particular, the blood infusion of the TRPV1 selective agonist, resiniferatoxin, triggered expression of immediate early gene *c-Fos* in astrocytes from circumventricular organs (Mannari et al. 2013).

6 TRPC Channels Coordinate Multi-ion Signalling in Astroglia

In astrocytes, out of all TRP proteins, the channels of TRPC family seem to be the most abundant, and are poised to have a specific functional importance in coordinating Ca^{2+} and Na^{+} signalling in response to widely heterogeneous stimuli (Fig. 2). First, TRPC channels are sensitive to mechanostimulation, which occurs quite frequently in astroglia that show a remarkable degree of morphological plasticity and are prone to rapid changes in their volume. These volume changes

can develop on a relatively rapid scale (seconds), accompanying, for example, synaptic transmission. Synaptic activity is directly associated with a transient local shrinkage of the extracellular space which is controlled by water transport across astroglial perisynaptic membranes mediated by AQP-4 with subsequent water redistribution through the glial syncytium (Haj-Yasein et al. 2012; Nagelhus et al. 2004). These local volume changes may activate TRPC channels with the subsequent initiation of local Na^+/Ca^{2+} signals; incidentally, activation of TRPC1 channels may regulate expression of AQP channels (Conner et al. 2012). Similarly, TRPC channels are activated during hypo-osmotic stress.

Second, TRPC1-containing channels (which predominantly mediate Na^+ fluxes) are under control of Ca^{2+} signalling machinery (being astroglial substrates for SOCE). As a result, it is plausible that metabotropic stimulation of astroglia that depletes ER Ca^{2+} stores would trigger opening of TRPC channels and induces substantial Na^+ fluxes (Fig. 2). This mechanism may translate activation of G-protein-coupled receptors into Na^+ signalling events developing in parallel with ER-mediated Ca^{2+} signals.

The role for TRPC channels in regulation of $[Na^+]_i$ and Na^+-dependent processes was first discovered in HEK cells in which TRPC3 protein appeared to be closely associated with NCX via the C-terminus of the channel. Interactions were reciprocal as the inhibition of NCX affected the Ca^{2+} flux through the TRPCs (Eder et al. 2005). In addition, a $[Na^+]_i$ elevation following the opening of TRPC channels led to a reversal of NCX with obvious consequences for Ca^{2+} signalling, that is, Ca^{2+} entry to the cytosol from the extracellular space. The role for TRPC-mediated Na^+ influx and resulting intracellular Na^+ signals in Ca^{2+}astrocytes could be even more important than that of Ca^{2+} dynamics, as astrocytes possess numerous molecular systems relevant for homeostatic responses that are controlled by the transmembrane Na^+ gradient (see (Kirischuk et al. 2012; Verkhratsky et al. 2013a) and Table 1).

TRPC channels seem to have a dual selectivity filter, as unveiled by site-directed mutagenesis and immunological approaches. Hence, substitution of seven acidic residues to basic amino acids in the channel region of TRPC1 subdued Ca^{2+}, but not Na^+ fluxes (Liu et al. 2003). Introduction of a single mutation (E630Q) to the selective filter of TRPC3 caused a reduction in Ca^{2+} current with a concomitant enhancement of Na^+ currents (Poteser et al. 2011). Having this in mind, Reyes et al. (2013) used a functional anti-TRPC1 antibody targeting the putative selective filter of the TRPC1 channel. As we already disclosed, mechanical stimulation of astrocytes triggers increases in both $[Ca^{2+}]_i$ and $[Na^+]_i$ (Fig. 1b and d, respectively, and (Malarkey et al. 2008; Reyes et al. 2013)). Inhibition of TRPC1 channels by the anti-TRPC1 antibody resulted in a decrease in the peak and cumulative $[Ca^{2+}]_i$ responses (Fig. 1b) and, in parallel, in an increase in the peak amplitude of $[Na^+]_i$ response (Fig. 1d) (Reyes et al. 2013). Taken together, Ca^{2+} and Na^+ fluxes of TRPC channels can thus be dissociated following molecular biology or immunological interventions. It is tempting to speculate that such mechanism of regulation of TRPC permeability could represent a physiological event, perhaps mediated by yet unknown enzymatic, protein-protein binding or post-translational modifications.

7 Pathological Potential of Astroglial TRP Channels

Astroglia, being the central homeostatic and defensive cellular elements of the CNS, are involved in the absolute majority of neurological diseases, and astroglial reactions to pathological insults to a great extent determine progression and outcome of neuropathology (Giaume et al. 2007; Verkhratsky et al. 2013b). Investigations of contribution and possible pathophysiological relevance of astroglial TRP channels are in statu nascendi with only several studies having been performed hitherto.

TRPA1 channels were detected in glial fibrillary acidic protein-positive astrocytes of the superficial laminae of the rat trigeminal caudal nucleus using electron microscopy in combination with immunohistochemistry and immuno-silver-gold labelling (Lee et al. 2012). Peripheral inflammation (induced by injection of complete Freund's adjuvant into the capsule of a temporomandibular joint) increased the number of labelled TRPA1 channels in astroglial processes contacting nociceptive primary afferent terminals of the joint. This was considered as an indication of a possible role for TRPA1 channels in the stimulation of astrogliotic reactions following activation of a nociceptive input.

The TRPC channels are implicated in Ca^{2+} signalling generated by thrombin that were demonstrated to be linked to initiation of astrogliotic remodelling (Nakao et al. 2008; Shirakawa 2012). On similar lines, astroglial Ca^{2+} signalling in response to acute administration of IL-1β results, in part, from activation of TRPC1 and TRPC6 channels, and chronic treatment with IL-1β increased TRPC6 expression that contributed to dysregulation of overall Ca^{2+} homeostasis (Beskina et al. 2007). TRPV4 channels also have been linked to astrogliotic response. Expression of TRPV4 channels in hippocampal astrocytes substantially increased following brief (15 min) episode of cerebral hypoxia/ischaemia produced by bilateral occlusion of the common carotid arteries together with systemic hypoxia (Butenko et al. 2012). This increased presence of TRPV4 channels resulted in an increase in respective ion currents and TRPV4-mediated Ca^{2+} signals. It has been also suggested that TRPV4 contributes to ischaemia-induced $[Ca^{2+}]_i$ elevations (Butenko et al. 2012). The TRPV4 channels were also implicated in astroglial cell death triggered by oxidative stress (Bai and Lipski 2010).

The TRP channels being multi-ion carriers can also be implicated in astroglial regulation of homeostasis of various metals and in metal-induced toxicity. The TRPC channels, for example, have been shown to participate in the buffering of iron, which ability increased in reactive cells (Pelizzoni et al. 2013). Astrocytes are also primary targets for the main forms of toxic encephalopathies induced by heavy metals. Accumulation of these metals in astroglia generally disrupts astroglial homeostatic abilities and often compromises astroglial glutamate uptake which in turn results in excitotoxic neuronal death. These astroglial impairments are central, for example, in poisoning by methylmercury or Minamata disease (Yin et al. 2007), lead toxic encephalopathy (De Keyser et al. 2008), manganese neurotoxicity (De Keyser et al. 2008) and aluminium toxic encephalopathy (Struys-Ponsar et al. 2000; Suarez-Fernandez et al. 1999). In part, accumulation of these metals into

astroglia is mediated by specific transporters. However, the role of TRP channels cannot be excluded, and this possible route for heavy metal entry has not been yet experimentally addressed.

8 Conclusions

Channels of TRP family are expressed in astroglia where they perform various, mainly yet undetermined functions in physiology and pathophysiology. The TRPC channels are uniquely placed to coordinate astroglial Ca^{2+} and Na^{+} signalling because of their Na^{+}/Ca^{2+} permeability and because of association of TRPC-mediated Na^{+}/Ca^{2+}influx with ER store depletion of releasable Ca^{2+}, which establishes a direct link between activation of metabotropic receptors and Na^{+} signalling.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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