

Mini-Symposium

New Insights on Astrocyte Ion Channels: Critical for Homeostasis and Neuron-Glia Signaling

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Initial biophysical studies on glial cells nearly 50 years ago identified these cells as being electrically silent. These first studies also demonstrated a large K^+ conductance, which led to the notion that glia may regulate extracellular K^+ levels homeostatically. This view has now gained critical support from the study of multiple disease models discussed herein. Dysfunction of a major astrocyte K^+ channel, Kir4.1, appears as an early pathological event underlying neuronal phenotypes in several neurodevelopmental and neurodegenerative diseases. An expanding list of other astrocyte ion channels, including the calcium-activated ion channel BEST-1, hemichannels, and two-pore domain K^+ channels, all contribute to astrocyte biology and CNS function and underpin new forms of crosstalk between neurons and glia. Once considered merely the glue that holds the brain together, it is now increasingly recognized that astrocytes contribute in several fundamental ways to neuronal function. Emerging new insights and future perspectives of this active research area are highlighted within.

Significance Statement

The critical role of astrocyte potassium channels in CNS homeostasis has been reemphasized by recent studies conducted in animal disease models. Emerging evidence also supports the signaling role mediated by astrocyte ion channels such as BEST1, hemichannels, and two-pore channels, which enable astrocytes to interact with neurons and regulate synaptic transmission and plasticity. This minisymposium highlights recent developments and future perspectives of these research areas.

Introduction

Next year (2016) marks the 50th anniversary of the first physiology studies examining glial cells (Kuffler et al., 1966; Orkand et al., 1966). These and other early seminal studies demonstrated

that glial cells rest at quite hyperpolarized resting membrane potentials relative to neurons (by >20 mV) and that glia display large and selective permeability to K^+ ions (Kuffler, 1967; Ransom and Goldring, 1973). Furthermore, due to the high selective K^+ permeability, the glial resting membrane potential was predicted by the Nernst equation for K^+ (Kuffler, 1967; Ransom and Goldring, 1973). These electrophysiological properties, the spatial arrangement in the CNS between astrocytes and neurons, and extensive astrocyte coupling via low-resistance intercellular pathways formed by gap junctions led to the formation of the K^+ spatial buffering hypothesis.

The biophysical characteristics described above are still used today by neurophysiologists to identify astrocytes within *in situ* and *in vivo* preparations during electrophysiological recordings. Work from multiple groups of investigators indicates that the large K^+ leak conductance reflects the intrinsic property of membrane ion channels and appears to be a general functional feature of mature astrocytes (Dallérac et al., 2013; Du et al., 2015). Astro-

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cytes exhibit low input resistances, often $<20\text{ M}\Omega$, as a result of the K^+ leak conductance, which represents a formidable barrier for the measurement of channel activity using traditional electrophysiological methods. This has proved particularly challenging for measuring ion flux at sites located distal to somata (Ma et al., 2014), particularly at perisynaptic and perivascular sites, where homeostatic support functions of K^+ channels are expected to contribute the most. The exact molecular signatures of the channels that orchestrate these membrane properties are unresolved, but appear to be due in part to the weakly rectifying K^+ channel Kir4.1 and two-pore domain K^+ channels. We discuss the latest findings of this critical channel in normal astrocyte and CNS function.

Work over the past two decades has led to multiple proposed pathways in astrocytes, such as Ca^{2+} -dependent exocytotic release of ATP and glutamate, that maintain and regulate synaptic transmission/plasticity (Panatier et al., 2011; Agulhon et al., 2012a; Araque et al., 2014; Haydon and Nedergaard, 2015). Meanwhile, a growing list of channels that are not K^+ selective but are expressed in astrocytes has been revealed. These channels regulate neurotransmission through various novel mechanisms, which is the focus of this discussion. We term this group of channels “signaling channels,” thus alluding to their primary involvement in astrocyte–neuron crosstalk. All of the signaling channels identified to date are poorly ion selective or nonselective cation/anion channels. We briefly discuss the latest developments on the calcium-activated anion channel bestrophin1 (Best-1), connexin 43 hemichannels, and the two-pore K^+ channel TWIK-1 and TREK-1 (Lee et al., 2010; Woo et al., 2012; Wang et al., 2013; Chever et al., 2014). Although not an exhaustive list, these channels have received recent attention regarding astrocyte–neuron communication in the context of normal CNS function and in the pathological brain and will be discussed below.

Kir4.1: an integral ion channel in astrocyte biology

Kir4.1 is a weakly inwardly rectifying K^+ channel, which in the nervous system is expressed exclusively in glial cells, with highest expression in astrocytes. In astrocytes, putative functions of Kir4.1 include K^+ homeostasis, maintenance of the astrocyte resting membrane potential, high astrocyte K^+ conductance, astrocyte cell volume regulation, and facilitation of glutamate uptake (Olsen et al., 2006; Dibaj et al., 2007; Djukic et al., 2007; Kucheryavykh et al., 2007; Seifert et al., 2009). Increased Kir4.1 expression and function are associated with astrocyte maturation (Seifert et al., 2009), whereas reduced channel expression and/or activity is associated with the CNS pathologies such as epilepsy (Ferraro et al., 2004; Scholl et al., 2009), Alzheimer’s disease (Wilcock et al., 2009), amyotrophic lateral sclerosis (Kaiser et al., 2006; Bataveljić et al., 2012), spinal cerebellar ataxia (Magaña et al., 2013), and pain (Vit et al., 2008). Pharmacological inhibition, knock-down, or complete knock-out of this channel results in astrocytes with increased membrane resistance, depolarized resting membrane potentials, and in extracellular potassium dynamics (Olsen et al., 2006; Kucheryavykh et al., 2007; Seifert et al., 2009; Chever et al., 2010; Sibille et al., 2015). Furthermore, studies using glia-specific conditional Kir4.1 knock-out mice or siRNA-mediated silencing of the gene indicate that loss of this ion channel results in aberrations in glutamate homeostasis (Djukic et al., 2007; Kucheryavykh et al., 2007), possibly by affecting astrocyte resting membrane potential or some other unknown direct/indirect interaction between Kir4.1 and glutamate transporters. In addition to cell-intrinsic glial cell dysfunction, loss of Kir4.1 affects neuronal function, as indicated by alterations in

hippocampal short-term plasticity (Sibille et al., 2014). Furthermore, ataxia, seizures, and early postnatal death (before postnatal day 25) in mice lacking Kir4.1 underscore the importance of the channel for normal CNS function. Mutations in the Kir4.1 gene (*KCNJ10*) are causative for the autosomal recessive disorder, SeSAME/EAST Syndrome (Seizures, Sensorineural deafness, Ataxia, Mental retardation and Electrolyte imbalance/Epilepsy, Ataxia, Sensorineural deafness and Tubulopathy) in human patients (Bockenbauer et al., 2009; Scholl et al., 2009). Neurological features of this syndrome include early onset seizures, ataxia, epilepsy, sensorineural hearing loss, cognitive impairments, and developmental delay. Furthermore, there exist subsets of patients diagnosed with autism and seizures who harbor mutations in the Kir4.1 channel (Sicca et al., 2011). These data suggest that glial Kir4.1 serves an important role in early CNS neuronal development and functioning.

Kir4.1 dysfunction in the neurodevelopmental disorder of Rett syndrome

Rett syndrome (RTT) is a devastating neurodevelopmental disorder, which is caused by spontaneous mutations in the X-linked transcriptional regulator methyl CpG binding protein 2 (MeCP2) in $>95\%$ cases (Neul et al., 2010). MeCP2 binds to methylated or unmethylated DNA to modulate gene transcription (Chahrouh et al., 2008). RTT is a leading cause of cognitive, motor, and communication impairment in females, affecting $\sim 1:10,000$ – $15,000$ female births worldwide each year. Loss of speech, seizures, ataxia, dystonia, stereotypies, and irregular breathing with hyperventilation/apnea while awake, difficulty swallowing, and pervasive growth failure, along with severe gastrointestinal disorders are just some of the problems that plague RTT patients (Neul et al., 2010). Although typically thought of as a neuronal disease, a recent study showed that reexpression of MeCP2 exclusively in astrocytes ameliorates or prevents overt symptoms in animal models of RTT (Lioy et al., 2011), demonstrating that astrocytes contribute significantly to the etiology of the disease. However, the mechanisms by which astrocytes contribute to RTT have not yet been identified.

RTT shares many common features with the SeSAME/EAST syndrome, which is caused by loss-of-function mutations in Kir4.1. The constellation of SeSAME/EAST syndrome neurological symptoms includes severe developmental delay, early onset seizures (~ 3 – 6 months of age), ataxia, lower motor extremity weakness, and sensorineural deafness (Scholl et al., 2009; Tang et al., 2010). The onset and early progression of neurological symptoms may be explained by reductions in functional Kir4.1, which typically demonstrates marked upregulation during late fetal and early postgestational development in human CNS (Kang et al., 2011; Fig. 1). Similar developmental increases in Kir4.1 mRNA and protein are observed in rodents (Poopalasundaram et al., 2000; Dibaj et al., 2007; Nwaobi et al., 2014). It was demonstrated that the transcriptional upregulation of *KCNJ10* is coincident with reductions in DNA methylation patterns of the gene. Manipulation of the methylation status of *KCNJ10* was sufficient to modify gene transcription (Nwaobi et al., 2014). As with many highly transcribed genes, the promoter region of *KCNJ10* is lowly methylated. Given that MeCP2 binds to lowly methylated gene promoters to activate gene transcription (Yasui et al., 2007), it was postulated that MeCP2 may directly regulate *KCNJ10* gene transcription. Supporting this, we observed a significant difference in Kir4.1 protein expression and function in a commonly used murine model of RTT (M. L. Olsen, V. A. Cuddapah, N. L. Pacheco, unpublished results). Interestingly, reduced Kir4.1 protein loss was observed early in postnatal development and before

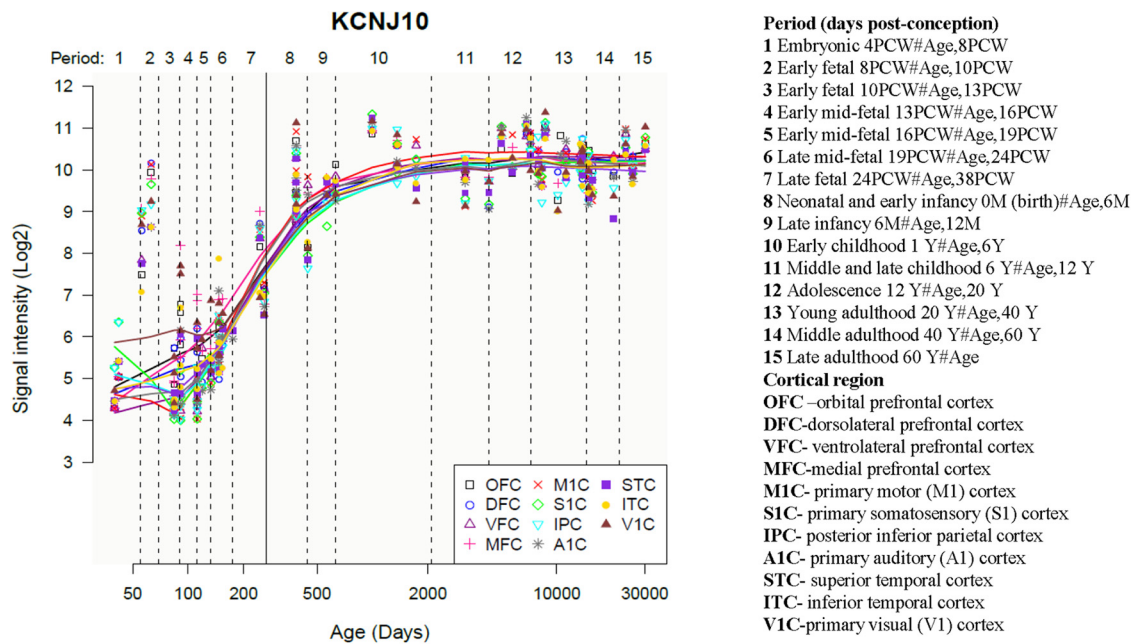


Figure 1. *KCNJ10* expression is upregulated during late fetal and early postnatal development. In human cortex, *KCNJ10* expression begins to show significant increase during late middle to late fetal development and continues to increase through early childhood. Expression stabilizes between early (<6 years of age) and middle to late childhood (12 years of age). Similar temporal patterns of expression were observed in amygdala, hippocampus, striatum, and medial dorsal nucleus of the thalamus (data not shown). Figure reprinted with permission (Kang et al., 2011).

the observation of RTT-like symptoms in mice. A ChIP assay revealed a direct interaction between the MeCP2 protein and the Kir4.1 gene promoter in WT mice that was absent in RTT mice. Together, these data suggest that MeCP2 positively modulates Kir4.1 gene transcription. Current studies are under way to determine whether alterations in astrocyte Kir4.1 expression and function play a direct role in RTT pathogenesis.

Dysfunction of astrocyte Kir4.1 occurs early on in Huntington's disease model mice

Huntington's disease (HD) is characterized by motor, cognitive, and psychiatric disturbances associated with neuronal dysfunction and atrophy of the striatum and other brain areas. HD is caused by an expanded chain of polyglutamines localized to the N-terminal region of the huntingtin protein that causes intracellular accumulation and aggregation of mutant huntingtin (Mangiarini et al., 1996). The molecular, cellular, and basal ganglia circuit mechanisms that produce disease phenotypes remain incompletely understood, although effort has focused on mutant huntingtin (mHTT) expression within neurons. Several recent studies suggest that astrocytes are also involved in HD (Shin et al., 2005; Bradford et al., 2009; Faideau et al., 2010), which extends data showing that brains from HD patients and from mouse models of HD have mHTT accumulation in striatal astrocytes (Shin et al., 2005; Faideau et al., 2010). Much remains unknown about how astrocytes contribute to HD pathogenesis.

Recently, it was discovered that Kir4.1 potassium ion channel expression was decreased in astrocytes that express mHTT (Tong et al., 2014), with little or no evidence for reactive astrogliosis at symptom onset in the transgenic R6/2 and knock-in Q175 mouse models of HD. The loss of a K^+ conductance depolarized astrocytes and medium spiny neurons *in vitro* and likely contributed to elevated levels of K^+ that were measured *in vivo* within the striatum of HD model mice (Tong et al., 2014). Moreover, rescuing the loss of astrocyte Kir4.1 reduced several deficits associated with HD mouse models (Tong et al., 2014). Unexpectedly,

restoring the loss of astrocyte Kir4.1 rescued astrocyte glutamate transporter Glt1 expression. This serendipitous observation was of relevance to the human disease because Glt1 levels are known to be reduced in HD and because Glt1 is heavily implicated in disease mechanisms (Liévens et al., 2001; Miller et al., 2008; Estrada-Sánchez and Rebec, 2012). Further detailed studies to explore signaling by astrocyte Kir4.1 channels, Glt-1 expression, and their functional relevance are needed. Nevertheless, the available data suggest that basal ganglia circuit level defects in HD and other brain disorders may be partly remedied by correcting key astrocyte dysfunctions that have ensuing consequences for neurons, microcircuits, and their outputs.

Regulation of Kir4.1 by intracellular spermine

Unlike typical voltage-gated ion channels, which gate in response to membrane depolarization, the pore of Kir4.1 is blocked at depolarized potentials by Mg^{2+} ions and polyamines. At or near the reversal potential for potassium (E_K), the block is removed and K^+ ions permeate the pore (Oliver et al., 1998; Tada et al., 1998). The direction of flux is determined by the electrochemical gradient for K^+ . Kir4.1, as originally described, was characterized as having intermediate rectification properties (Tada et al., 1998). An attractive idea put forth in this study was that features of channel rectification would allow for both K^+ influx and efflux through the same channel, thus making this channel ideally suited for the regulation of K^+ concentrations. The rectification properties of Kir4.1, like all other inward-rectifying K^+ channels, are governed by intracellular polyamines (Guo and Lu, 2002). (Oliver et al., 1998). Polyamines such as spermine are ubiquitous biogenic molecules essential for all mammalian cells (Pegg, 2014). Spermine is implicated in many cellular processes ranging from transcription to nucleic acid stability. Intriguingly, spermine is selectively accumulated in astrocytes (Laube and Veh, 1997) and physiological concentration of polyamine is crucial for the opening status of connexin43 (Skatchkov et al., 2000;

Skatchkov et al., 2014). Therefore, the polyamine spermine may be critical for the integrative function of astrocytes at the syncytial level. For example, holding the cytoplasmic concentration of spermine at 1 mM, a physiologically relevant concentration (Kucheryavykh et al., 2008; Skatchkov et al., 2014), while applying 10–100 μ M glutamate externally to activate Na^+ -dependent glutamate uptake resulted in an increase of intracellular Na^+ concentration of \sim 10 mM and a switch of K^+ currents from weakly to strongly rectifying in both cortical astrocytes and retinal Müller glia (Kucheryavykh et al., 2008). These data suggest that Na^+ , such as through glutamate transporters, can optimize glial Kir4.1 function. Interestingly, polyamine biosynthesis and concentrations vary during cell cycle progression (Oredsson, 2003) and differing metabolic demands on the cell (Kauppinen and Alhonen, 1995). It is conceivable that the gating properties of Kir4.1, and therefore flux through the channel in astrocytes, are dependent on parameters that govern astrocyte polyamine and Na^+ concentrations. Given that Kir4.1 is expressed in astrocytes throughout the CNS, intracellular polyamine and Na^+ concentrations may permit astrocytes to customize ion flux according to regional demands.

Mutations in the Kir4.1 gene, *KCNJ10*

As mentioned above, mutations in the Kir4.1 gene, *KCNJ10*, are causative for SeSAME/EAST syndrome in human patients. The predicted structure of a single Kir4.1 subunit, the identified mutations, and the respective amino acid changes are shown in Figure 2. Deficits in channel function were revealed using heterologous expression systems for each identified mutation. Homomeric expression of five mutations identified in the human population (R199Stop, C140R, T164I, G77R, and R297C) revealed a near complete loss of function at physiological pH (Sala-Rabanal et al., 2010; Tang et al., 2010). In contrast, R65P and A167V mutants conducted K^+ ions, but significantly less so than WT *KCNJ10* (Sala-Rabanal et al., 2010). Coexpression with the Kir5.1 subunit, which is thought to form heteromeric channels with Kir4.1 in some instances, did not rescue channel dysfunction of Kir4.1 mutants. In contrast, channel function was rescued with coexpression with wild-type Kir4.1 channels (Sala-Rabanal et al., 2010), suggesting that, in humans, Kir4.1 is composed of homomeric subunits.

Channels with signaling functions in astrocytes

Functions for signaling channels range from regulation of brain development to synaptogenesis, synaptic transmission and plasticity, and functional neuron-astrocyte-vascular coupling (Lalo et al., 2011; Illes et al., 2012; Scemes and Spray, 2012; Filosa et al., 2013; Verkhratsky et al., 2014).

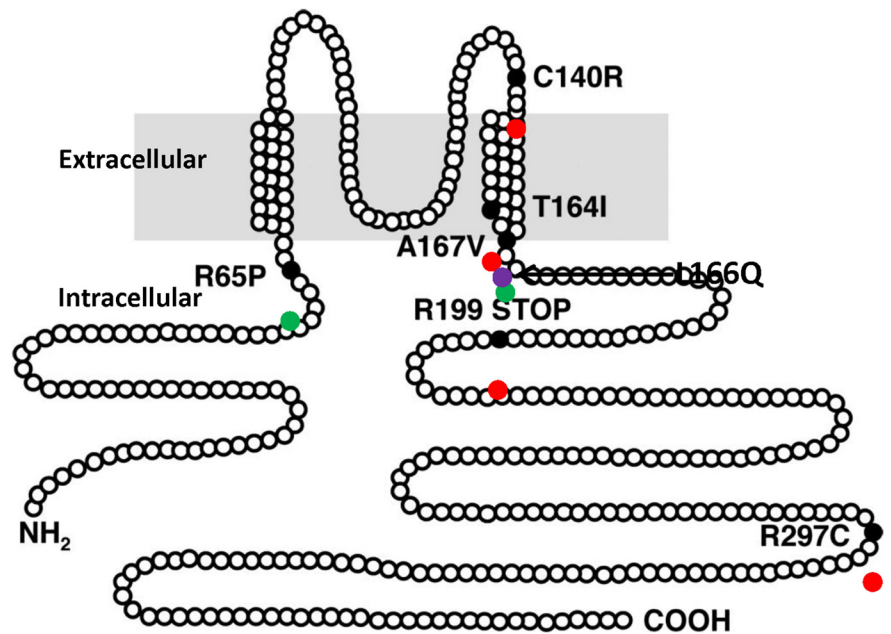


Figure 2. Location of *KCNJ10* mutations in patients with SeSAME syndrome. A schematic view of the protein is shown, with intracellular N and C termini, two transmembrane helices (plasma membrane shown in shaded gray), and one pore. This structure is characteristic of the inward rectifier family. Locations of mutations that were nonconducting are indicated by red circles and reduced conduction are indicated by green circles. L166Q is a human single nucleotide polymorphism identified by PolyPhen analysis as being “probably damaging” (purple circle). Homozygous expression of this channel resulted in reduced K^+ currents and K^+ uptake. The respective amino acid change for each mutation is noted. Figure adapted with permission (Scholl et al., 2009).

TREK-1 as a glutamate release channel and TWIK-1 as a sensor of glutamatergic transmission

In the quest for a full understanding of K^+ channel expression profiles, TWIK-1 and TREK-1, two of the two-pore domain K^+ channel (K_{2p}) isoforms, came into the focus as promising candidates (Fig. 3; Cahoy et al., 2008). Interestingly, membrane TREK-1 provides a fast glutamate release pathway upon activation of $G_{\alpha i}$ coupled GPCR, and glutamate release is triggered by interaction of dissociated $G\beta\gamma$ with the N terminus of TREK-1 channels (Woo et al., 2012). However, whether TWIK-1 contributes as a classic K^+ channel to the astrocyte’s passive conductance is not yet resolved (Zhou et al., 2009; Wang et al., 2013; Hwang et al., 2014). Interestingly, this channel has attracted attention because of its peculiar ion conduction and trafficking features. Similar to kidney tubular and pancreatic β cells, a large amount of TWIK-1 is retained in intracellular compartments. When trafficked to the plasma membrane, TWIK-1 is a nonselective monovalent cation channel (Millar et al., 2006; Chatelain et al., 2012; Wang et al., 2013). Recent work has demonstrated that TWIK-1 inward NH_4^+ currents were 40-fold larger than those carried by K^+ (Ma et al., 2012), implying that TWIK-1 is an efficient NH_4^+ uptake channel in astrocytes. In addition, membrane TWIK-1 expression can be regulated by G_i/G_o -coupled receptors such as the 5-HT and α_2 -adrenoceptor receptors (Felicangeli et al., 2010). The G_i -coupled metabotropic glutamate receptor 3 (mGluR3) is the predominant mGluR isoform in cortical and hippocampal astrocytes in adult mice (Sun et al., 2013; Hausteiner et al., 2014). This places mGluR3 as the predominant metabotropic glutamate receptor expressed in hippocampal astrocytes sensing dynamic changes in glutamatergic synaptic transmission, which occur under physiological and pathological conditions in the CNS. Both proteins, mGluR3 and TWIK-1, are highly expressed in astrocytes, prompting the hypothesis that activation of

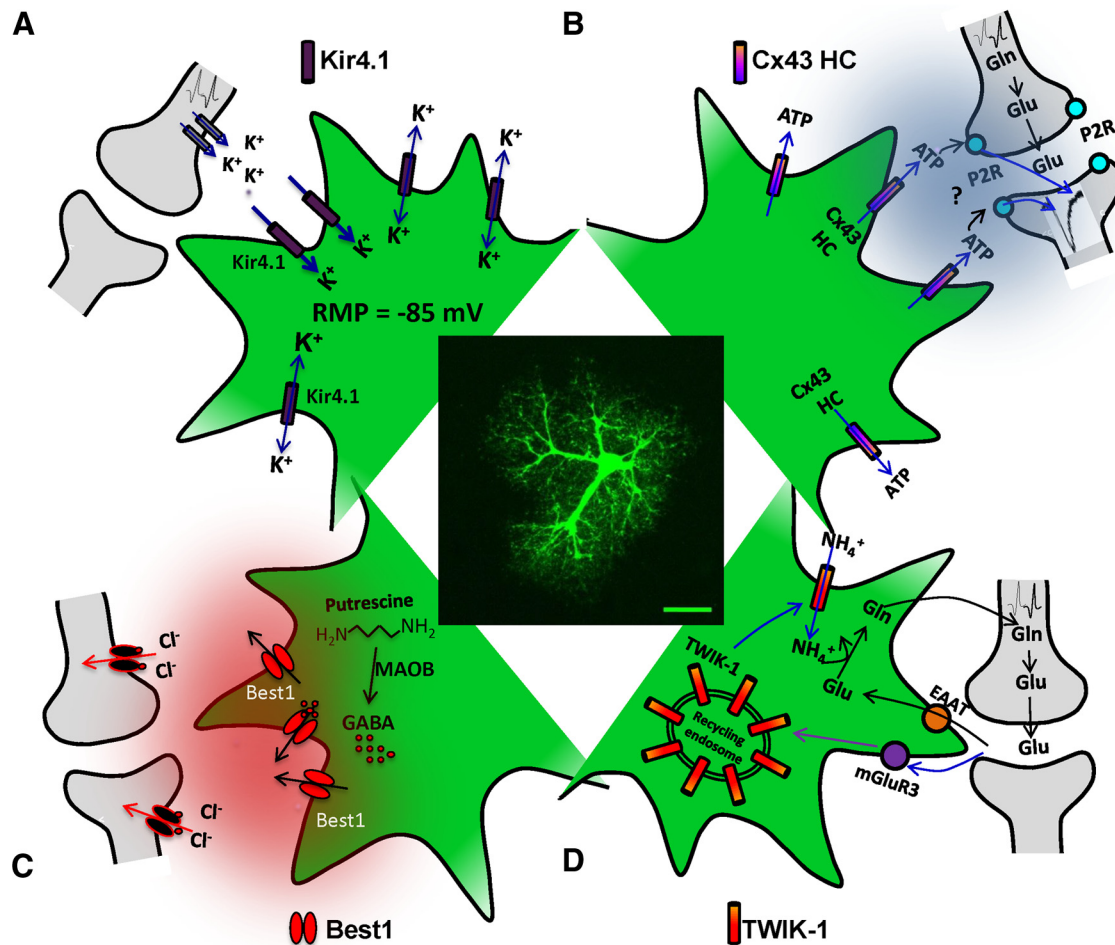


Figure 3. Role of astrocyte K^+ and signaling channels. Shown is a confocal image of a hippocampal astrocyte *in situ* revealed by intracellular loading of Alexa Fluor-488 (courtesy of Jonathan Zapata). Scale bar, 20 μm . **A**, Kir 4.1 functions as a major K^+ channel establishing hyperpolarized astrocyte membrane potential and spatially redistributing the K^+ concentration. **B**, Cx43 hemichannels modulate basal glutamatergic synaptic activity through ATP signaling. In basal conditions, Cx43 hemichannels boost hippocampal excitatory postsynaptic activity through ATP signaling. A possible scenario is that Cx43 hemichannels release ATP, which activates P2 receptors on CA1 pyramidal cells that increase glutamatergic postsynaptic activity. **C**, Astrocytic GABA is produced via putrescine degradation pathway with a key biosynthetic enzyme, MAOB. GABA release from Best1 contributes significantly to the tonic GABA release under physiological and various pathologic conditions. **D**, Activation of mGluR3 translocates TWIK-1 from recycling endosome to membrane that enhances NH_4^+ uptake, which may facilitate glutamine–glutamate cycle for replenishment of neurotransmitters in neurons.

mGluR3 recruits cytoplasmic TWIK-1 channels to membrane in hippocampal astrocytes (W. Wang, C. M. Kiyoshi, Y. Du, B. Ma, M. Zhou, unpublished observation). Activation of mGluR3 increased membrane TWIK-1 expression through a Rab-mediated endosomal recycling pathway. Functionally, increased membrane TWIK-1 expression was associated with elevated NH_4^+ uptake. Therefore, neuronal glutamate release may regulate TWIK-1 membrane expression and NH_4^+ uptake dynamically in astrocytes, which in turn provide glutamine for neurotransmitter renewal through the glutamate–glutamine cycle.

Best1's role in tonic GABA release and neurological diseases

Bestrophin (*Best*) is the gene responsible for a dominantly inherited, juvenile-onset form of macular degeneration called Best vitelliform macular dystrophy. It has been shown to encode a functional Ca^{2+} -activated anion channel that is activated directly by submicromolar intracellular Ca^{2+} in nonneuronal tissues and peripheral neurons (Hartzell et al., 2008). Several studies have suggested the function of Best in various physiological processes such as normal vision and cell volume regulation (Hartzell et al., 2008; Milenkovic et al., 2015). In the brain, Best1 was found to be

largely expressed in astrocytes (Park et al., 2009; Oh et al., 2012), especially at the microdomains near synaptic junctions (Woo et al., 2012; Park et al., 2013). As an ion channel, Best1 shows a relatively high permeability to large anions, including glutamate and isethionate, along with chloride ions (Park et al., 2009; Park et al., 2013). It has been demonstrated that astrocytes release glutamate via Best1 (Woo et al., 2012), which then targets and activates synaptically localized, GluN2A-containing NMDA receptors in hippocampal CA1 pyramidal neurons (Han et al., 2013) to modulate hippocampal synaptic plasticity (Park et al., 2015).

In addition to glutamate, Best1 has a significant permeability to gamma aminobutyric acid (GABA) and mediates its tonic release from astrocytes in cerebellum to strongly inhibit neuronal excitability (Lee et al., 2010). GABA release from astrocytes may contribute significantly to tonic release of GABA observed in many brain regions under physiological conditions, as well as under various pathological conditions. Tonic inhibition is highly correlated with the presence of cytosolic GABA in cerebellum and hippocampus and other brain regions (Yoon et al., 2011); tonic inhibition was high and immunostaining for GABA in astrocytes was high in cerebellum, whereas tonic inhibition was low and

immunostaining for GABA was low in astrocytes in CA1 hippocampus. Astrocytic GABA is produced via putrescine degradation pathway with monoamine oxidase B (MAOB) as a key biosynthetic enzyme (Yoon et al., 2014). Therefore, under physiological conditions, astrocytic GABA is synthesized by MAOB and released by Best1 when there are sufficient levels of cytosolic GABA.

Astrocytic GABA is elevated in pathological conditions such as Alzheimer's disease. Reactive hippocampal astrocytes near amyloid plaques contain high levels of GABA (Jo et al., 2014; Wu et al., 2014), show elevated putrescine and use MAOB to produce GABA (Jo et al., 2014). Because reactive astrocytes in Alzheimer's disease model mice showed elevated Ca^{2+} levels (Kuchibhotla et al., 2009), the synthesized GABA was readily released through Best1 (Jo et al., 2014). Interestingly, elevated GABA is associated with altered distribution patterns of Best1, from a microdomain-specific to soma- and process-specific expression patterns (Jo et al., 2014). These results demonstrate the dynamic nature of Best1: the functional switch and differential trafficking from physiological conditions to pathological conditions and from glutamate releasing at synaptic junction to tonic GABA release at soma and processes. In conclusion, Best1's role in GABA release may be important in the context of gliosis observed in neurodegenerative diseases.

Astrocyte connexin hemichannels shape basal synaptic transmission

Astrocytes express high level of connexins (Cx), the protein subunits forming gap junction channels that mediate the extensive astrocyte network communication (Pannasch and Rouach, 2013). Each gap junction channel is formed by the alignment of two hemichannels (HCs), and the connexons are composed of six transmembrane proteins. In astrocytes, the primary connexons are Cxs 43 and 30. Astrocyte Cxs contribute to synaptic strength or memory in physiological conditions, notably by regulating neurometabolic coupling (Rouach et al., 2008), extracellular homeostasis (Wallraff et al., 2006; Pannasch et al., 2011), astrocyte synapse coverage (Pannasch et al., 2014), or gliotransmitter release (Stehberg et al., 2012; Torres et al., 2012; Chever et al., 2014). In contrast to gap junctions, astrocyte hemichannels mediate direct exchange with the extracellular space and, remarkably, Cxs also demonstrate channel-independent functions involving protein interactions and/or cell adhesion (Theis et al., 2005; Elias and Kriegstein, 2008; Pannasch and Rouach, 2013; Pannasch et al., 2014). Cx HCs represent an important pathway of release or uptake of ions and signaling molecules in astrocytes (Giaume et al., 2013; Cheung et al., 2014). Initially, Cx HCs were thought to be activated primarily during pathological situations because their opening was viewed as deleterious by inducing loss of cytoplasmic integrity and neurotoxic damage (Giaume et al., 2013; Cheung et al., 2014). However, several recent studies suggest emerging physiological functions that include the regulation of synaptic activity and memory through gliotransmitter release (Stehberg et al., 2012; Torres et al., 2012; Cheung et al., 2014; Chever et al., 2014).

Astrocyte Cx43 HCs are open under resting conditions in acute hippocampal slices and tune basal glutamatergic synaptic transmission via ATP signaling (Chever et al., 2014). To investigate whether such opening of Cx43 HCs regulates basal synaptic transmission, EPSCs from hippocampal CA1 pyramidal cells were investigated. A rapid decrease in EPSCs was seen upon acute inhibition of Cx43 HCs with the mimetic blocking peptide Gap26, indicating that astrocyte Cx43 HCs endogenously

strengthen basal glutamatergic synaptic transmission. Remarkably, basal extracellular ATP levels, measured locally and in real time in hippocampal slices using a luciferin–luciferase luminescence assay, were also concurrently decreased by the Gap26-blocking peptide, suggesting that ATP is released tonically through Cx43 HCs in resting conditions. To investigate whether ATP release by Cx43 HCs modulates excitatory synaptic transmission in hippocampal slices through direct activation of ATP purinergic receptors, the ATP P2X and P2Y receptors were acutely blocked. This treatment mimicked the decreased excitatory synaptic transmission induced by Gap26 inhibition of Cx43 HCs and also occluded the effect of Gap26 on synaptic transmission. Basal extracellular ATP levels resulting from Cx43 HC-mediated ATP release thus exert a tonic facilitation of hippocampal excitatory transmission by direct activation of ATP purinergic receptors. Together, these data reveal Cx43 HCs as a novel physiological pathway of ATP release strengthening the moment-to-moment excitatory synaptic transmission in the hippocampus.

Conclusions

Astrocytes are the most numerous cells in the CNS. They tile the entire brain and serve vital trophic, active, and homeostatic roles (Barres, 2008). Growing evidence indicates that astrocytes contribute to neurological and psychiatric disorders (Maragakis and Rothstein, 2006; Barres, 2008; Ilieva et al., 2009; Nedergaard et al., 2010; Agulhon et al., 2012b; Clarke and Barres, 2013). However, key questions about astrocyte engagement in neural circuits, such as whether active or homeostatic roles dominate, remain unclear. Overall, deciphering the precise settings under which astrocytes contribute to neuronal circuit function in health and disease has proven challenging (Khakh and McCarthy, 2015) and a clearer understanding of the critical processes mediated by this cell type will illuminate how the healthy CNS functions and provide insight into pathological disease mechanisms.

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