JCMM
Available online at
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www.jcmm.org
Neuroscience Review Series

J. Cell. Mol. Med. Vol 10, No 1, 2006 pp. 33-44

Guest Editor: E.C. Toescu

Inwardly rectifying potassium channels (Kir) in central nervous system glia: a special role for Kir4.1 in glial functions

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Received: December 13, 2005; Accepted: February 15, 2006

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Abstract

Glia in the central nervous system (CNS) express diverse inward rectifying potassium channels (Kir). The major function of Kir is in establishing the high potassium (K^+) selectivity of the glial cell membrane and strongly negative resting membrane potential (RMP), which are characteristic physiological properties of glia. The classical property of Kir is that K^+ flows inwards when the RMP is negative to the equilibrium potential for K^+ (E_K), but at more positive potentials outward currents are inhibited. This provides the driving force for glial uptake of K^+ released during neuronal activity, by the processes of "K^+ spatial buffering" and "K^+ siphoning", considered a key function of astrocytes, the main glial cell type in the CNS. Glia express multiple Kir channel subtypes, which are likely to have distinct functional roles related to their differences in conductance, and sensitivity to intracellular and extracellular factors, including pH, ATP, G-proteins, neurotransmitters and hormones. A feature of CNS glia is their specific expression of the Kir4.1 subtype, which is a major K^+ conductance in glial cell membranes and has a key role in setting the glial RMP. It is proposed that Kir4.1 have a primary function in K^+ regulation, both as homomeric channels and as heteromeric channels by co-assembly with Kir5.1 and probably Kir2.0 subtypes. Significantly, Kir4.1 are also expressed by oligodendrocytes, the myelin-forming cells of the CNS, and the genetic ablation of Kir4.1 results in severe hypomyelination. Hence, Kir, and in particular Kir4.1, are key regulators of glial functions, which in turn determine neuronal excitability and axonal conduction.

Keywords: Kir • inward rectifying potassium channel • Kir4.1 • glia • astrocyte • oligodendrocyte • myelin resting membrane potential • potassium spatial buffering

Kir are key regulators of glial function

A high selective membrane permeability to potassium ions (K^+) and a strongly negative resting membrane potential (RMP) are considered fundamental properties of glial cells [1, 2]. Glia express a wide range of K^+ channels, but inwardly rectifying K^+ channels (Kir) are predominantly responsible for the high K^+ permeabili-

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Reprinted from:
Journal of Cellular and Molecular Medicine

Published by:
the CMM Foundation

ty and maintaining the RMP close to the equilibrium potential of potassium (E_K) [3–5]. Electrophysiological studies have demonstrated that Kir are the main K^+ conductance in the major macroglial cell types, namely astrocytes and oligodendrocytes, as well as retinal Müller glia and cerebellar Bergmann glia [3–10], although electrophysiological studies demonstrate that astrocytes are heterogeneous with respect to Kir currents and RMPs [11–15]. Kir play a key role in glial regulation of extracellular potassium concentration ($[K^+]_o$) by the processes of "K^+ spatial buffering" or "K^+ siphoning" [2, 4, 6–8]. Potassium regulation is generally considered a primary function of astrocytes and retinal Müller glia, in which K^+ released during neuronal activity enters glia *via* Kir and is 'spatially buffered' through the glial syncytium or 'siphoned' from areas of high $[K^+]_o$ to areas of low $[K^+]_o$, such as blood vessels [6]. The strict dependence of Kir on voltage and $[K^+]_o$ allows greater K^+ uptake when $[K^+]_o$ is raised [6–8]. In the brain, K^+ is redistributed through the astroglial syncytium *via* the extensive cell coupling and gap junctional communication between astrocytes [16]. Notably, in addition to their role in K^+ buffering, there is evidence that a developmental increase in Kir and a negative shift in the RMP are critical for oligodendrocyte differentiation and the formation of myelin [17–21]. It is therefore of considerable interest to determine the specific subtypes and functional properties of Kir expressed by CNS glia. In this paper, we review the evidence that glial cells express multiple Kir with diverse biophysical properties. An important feature in the CNS is the widespread and glia-specific expression of the Kir4.1 channel subtype, which has a special role in the fundamental glial functions of K^+ regulation and myelination.

The Kir channel family

The Kir channel family is generally divided on the basis of molecular and electrophysiological attributes into seven subfamilies (Kir1.0-Kir7.0), which have more than 20 members [22–28]. Furthermore, Kir channels are tetrameric structures and can be formed by co-assembly of heteromeric or homomeric Kir subunits, which endows the channels with distinct properties and further increases their functional diversity [29–34]. The defining features of Kir channels are their ability to conduct better in the inward direction than the outward direction, steep voltage dependence, a strict dependence on $[K^+]_o$, and strong modulation by intracellular factors and second messengers [22–26]. Potassium ions flow through inward rectifier channels when the RMP is negative to the equilibrium potential for K^+ (E_K), but at more positive potentials there is a voltage-dependent block of outward currents by intracellular polyamines, most potently by spermine, and Mg^{2+} [26]. Spermine switches off the K^+ conductance when the RMP is more positive than E_K, but the spermine block is strongly dependent on $[K^+]_o$, whereby the block decreases and channel conductance increases as the $[K^+]_o$ increases. Thus, Kir set the RMP close to the E_K, a characteristic of glia and critical for many of their functions.

Functionally, Kir channels can be divided into 5 subtypes on the basis of their biophysical properties [23, 24, 27, 28], namely: (1) classical inward rectifying K^+ channels, Kir2.1 (IRK1), Kir2.2 (IRK2), and Kir2.3 (IRK3); (2) G protein-activated K^+ channels, Kir3.1 (GIRK1), Kir3.2 (GIRK2), Kir3.3 (GIRK3), and Kir3.4 (GIRK4/CIR); (3) ATP-sensitive K^+ channels, Kir6.1 (uK_{ATP}-1), and Kir6.2 (BIR); (4) ATP-dependant K^+ channels, Kir1.1a (ROMK1/K_{AB}-1) and Kir4.1 (K_{AB}-2/BIR10); and (5) others, including Kir5.1 (BIR9), and tandem two-pore (2P) domain weak inward rectifying K^+ channel (TWIK)-related K^+ channels (TREK1,2) and TWIK-related acid-sensitive K^+ channels (TASK1-5). Their functional diversity is increased further by the formation of heteromeric channels [29–34]. Functional Kir3.0 channels are generally formed by co-assembly of Kir3.1 subunits with other members of the same family, such as the Kir3.1/Kir3.4 heteromers in atrial myocytes, which are responsible for the acetylcholine (ACh)-induced deceleration of heart beat [29, 30]. Co-assembly of Kir3.0 and Kir4.1 subunits has been described, but they are generally not viable or rapidly degrade [30, 31]. In contrast, heteromeric co-assembly of Kir4.1 with Kir2.1 results in a channel that is more strongly rectifying than homomeric Kir4.1 channels [32]. Kir5.1 subunits do not form functional homomeric channels, and exhibit highly selective heteromultimerization with Kir4.1 to generate heteromeric Kir4.1/Kir5.1 channels with unique rectification and kinetic properties [33, 34]. Kir5.1 also co-assembles with Kir2.1 to form electrically silent channels, thus negatively controlling Kir2.1 channel activity in native cells [35].

The distinct biophysical properties of the different Kir enable them to fulfil a diverse range of functions. The Kir2.0 family is constitutively active and widespread in excitable cells, where they are strongly rectifying and contribute to setting the RMP close to the E_K and repolarisation of the action potential [24, 25, 28]. The archetypal Kir3.0 channel is the ACh-activated K^+ channel (K_{ACh}) in cardiac myocytes, which mediate the slowing of the heart rate and can be activated by ACh acting on muscarinic receptors and adenosine acting on A1-purinergic receptors [24, 28, 29]. In addition to ACh and adenosine, Kir3.0 channels in the nervous system are coupled to a range of G-protein linked neurotransmitter receptors, including $GABA_B$, α2-adrenergic and serotonergic receptors [24, 28, 36, 37]. The defining attribute of Kir6.0 channels is that they are closed by micromolar concentrations of intracellular ATP, and serve to hyperpolarise cells by the loss of K^+ during metabolic inhibition, although neuronal K_{ATP} are only closed by millimolar concentrations of ATP and their function is less clear [22, 23, 38]. The ATP-dependent Kir1.1 and Kir4.0 channels, and heteromeric Kir5.1/Kir4.1 channels, are key channels in K^+ transporting cells, including kidney, stomach and glia, where they are responsible for cellular uptake of K^+ uptake and supplying K^+ to Na^+-K^+ pumps and proton pumps [24, 39–41]. Two-pore domain TREK and TASK channels are an emerging family of K^+ channels and are implicated in the control of the RMP and membrane excitability, ion and water transport, acid-base homeostasis, and hormone secretion, and are expressed highly in heart, smooth muscle, stomach, kidney, pancreas and the CNS [42, 43]. These channels are strongly regulated by hormones and neurotransmitters, and can be activated by temperature, membrane stretch and internal acidosis [42–44]. TREK-1 is myogenic in heart muscle [45] and smooth muscle [46], and has a protective function in neurons [47]. TASK-1, TASK-2, and TASK-3 have been shown to enhance neuronal viability by inhibiting the activation of intracellular apoptosis pathways [48], but in another study the activity of TASK-3 was responsible for K^+-dependent apoptosis of cultured cerebellar granule neurons [49]. Glia can express representatives of most if not all subtypes of Kir, and the properties of these channels in diverse cells indicate the multiple functions they are likely to have in glia, including setting the RMP, resistance to hypoxia, control of excitability, K^+ and H^+ transport, cell volume regulation, and cell survival.

Glial Kir

The most complete studies of glial Kir expression are on Müller glia, but a smaller number of studies on astrocytes and oligodendrocytes concur they express representatives of all the Kir subtypes (Table 1). The Kir4.1 subtype is an almost exclusively glial channel in the CNS, and has been demonstrated in astrocytes, oligodendrocytes, cerebellar Bergmann glia, and retinal Müller glia [50–60]. The literature is in general agreement that astrocytes express Kir4.1, but there have been contradictory reports concerning Kir4.1 in white matter astrocytes and oligodendrocytes. Higashi and colleagues [59] were unable to detect staining in white matter astrocytes or oligodendrocytes, but we and others have shown that astrocytes and oligodendrocytes are strongly immunopositive for Kir4.1 in white matter and grey matter (Fig. 1) [52, 55, 60]. Moreover, a critical role for Kir4.1 has been demonstrated in oligodendrocytes in Kir4.1 knock-out mice [20, 61–63]. It is clear that there is widespread expression of Kir4.1 in both oligodendrocytes and astrocytes, although there is likely to be heterogeneity both within and between CNS regions [52].

Interestingly, astrocytes and Müller glia also express Kir5.1 [58, 59], which do not form functional homomeric channels, but form heteromeric channels by a specific co-assembly with Kir4.1 [24, 33]. To the authors' knowledge, it is not known whether oligodendrocytes express Kir5.1 *in situ*. In addition, glia also express strongly rectifying Kir2.0 channels. Retinal Müller glia express mRNA for Kir2.1, Kir4.1 and Kir5.1, as well as Kir2.2, Kir2.4 [63], and are immunopositive for Kir2.1, Kir4.1 and Kir5.1 [56, 58]. Hippocampal astrocytes express message for Kir2.1, Kir2.2, and Kir2.3 [53, 57], and immunolabelling for Kir2.2 has been demonstrated in Bergmann glia and cerebellar astrocytes [64], whilst astrocytes and oligodendrocytes of the forebrain and hindbrain displayed heterogeneous labelling for Kir2.1, Kir2.2

Table 1 Functional Characteristics of the main Kir expressed by Glia

Subfamilies	Sub-types	Other Names	Functional Characteristics	Heteromeric associations
(1) Classical Rectifying K^+ Channels	Kir2.1 Kir2.2 Kir2.3	IRK1 IRK2 IRK3	- constitutively active - strongly rectifying - modulated by $[K^+]_o$ - gating by intracellular spermine and Mg^{2+} - regulated by intracellular PKA (stimulation) and PKC (inhibition)	- Kir2.1/5.1 (see below) - Kir2.1/4.1 (see below)
(2) G-Protein Activated K^+ Channels	Kir3.1 Kir3.2 Kir3.3 Kir3.4	GIRK1 GIRK2 GIRK3 GIRK4/CIR	- G-protein linked to a number of receptors - classical cardiac K(ACh) -activated by ACh muscarinic receptors - adenosine A1 receptors - also - dopamine D_2, $\alpha 2$ adrenergic, $GABA_B$, serotonergic, opioid	- formed of heteromers amongst Kir3.0 - mainly Kir3.1 with other Kir3.0 - K(ACh) are Kir3.1/3.4 heteromers
(3) ATP-Sensitive K^+ Channels	Kir6.1 Kir6.2	KATP-1 BIR	- weakly rectifying - closed by μM $[ATP]_i$ (only active when ATP very low) - but in neurones closed by mM ATP (not active at physiological ATP levels) - spermine block highly sensitive to pH_i (less block at acidic pH) - regulated by PIP_2	- four Kir6.0 units co-assemble with four sulphonylurea receptors (SUR1 and SUR2)
(4) ATP-dependent K^+ Channels	Kir4.1	KAB-2 BIR10	- weakly rectifying as homomeric channels - strongly rectifying when heteromeric with Kir2.1 or Kir5.1 - highly sensitive to pH_i and $[K^+]_o$	- form preferential heteromers with Kir5.1 - Kir4.1/2.1 - Kir4.1/3.0 not stable
(5) Others	Kir5.1	BIR9	- do not form functional homomeric channels - strongly rectifying when heteromeric with Kir4.1 - highly sensitive to CO_2/pH_i	- highly specific co-assembly with Kir4.1 - assembly with Kir2.1 forms electrically silent channels
(6) TWIK-related K^+ channels	TREK-1 TREK-2 TASK-1 TASK-2 TASK-3		- weakly rectifying - sensitive to pH_o and pH_i - strongly regulated by hormones and neurotransmitters - activated by anaesthetics - stretch activated	

Abbreviations: ACh - actetyl choline; AP - action potential; AQP4 - aquaporin 4; $[ATP]_i$ - intracellular ATP concentration; E_K - equilibrium potential for K^+; EP - electrophysiology (patch-clamp); IHC - immunohistochemistry;

Distribution	Functions	Glial Expression	Refs
- widespread in excitable cells - heart - neurons	- set the RMP close to the E_K - repolarisation of AP - shape of cardiac AP	**Widely expressed:** Muller glia mRNA: 2.1, 2.2 IHC: 2.1 Astrocytes mRNA: 2.1, 2.2, 2.3 IHC: 2.1, 2.2, 2.3 Oligodendrocytes IHC: 2.1, 2.2, 2.3 Bergmann glia IHC: 2.2	 60 55, 57 52, 56 61-64 62 61
- heart muscle - specific populations of neurons	- K(ACh) slow the heart rate - modulate neurotransmitter responses in neurons - decrease membrane excitability by hyperpolarising RMP	**Little evidence:** Muller glia mRNA: 3.1, 3.2 IHC: negative for 3.1, 3.2, 3.3, 3.4 Astrocytes IHC: 3.1 *in vitro* Oligodendrocytes EP: 5HT, ACh inhibit GIRK	 60 65 64 76, 77
- widespread in excitable cells - heart muscle - skeletal muscle - smooth muscle - neurons - renal tubule - pancreatic β cells	- maintain RMP during metabolic inhibition - regulation of neuronal excitability and neurotransmitter release - links insulin secretion to metabolic state in pancreatic β-cells	**Variable expression:** Muller glia mRNA: 6.1, 6.2 IHC: 6.1, SUR1 Astrocytes mRNA: 6.2, SUR1 IHC: 6.1, 6.2 Oligodendrocytes mRNA: 6.2 IHC: 6.1, 6.2 Bergmann glia IHC: 6.1	 60 65–67 68 69 70 69, 71 71
- key channels in transporting cells - renal tubules (Kir4.1/5.1) - stomach (Kir4.1/2.1) - glia	- cellular uptake of K^+ - supplying K^+ for Na-K pumps and proton pumps - water transport in association with AQP4	**Highly specific, widespread and strong expression:** Muller glia mRNA, IHC, EP Astrocytes mRNA, IHC, EP Oligodendrocytes mRNA, IHC, EP Bergmann glia IHC	 10, 50, 55, 57 , 60 51–54, 56, 58, 59, 60 20, 51, 59, 60 51
- renal tubules - central chemoreceptorneurons - glia	- link K^+ and H^+ transport - CO_2 sensitivity in chemoreceptor neurons	**Strong expression:** Muller glia mRNA, IHC Astrocytes IHC	 55, 57, 61 58
- widely expressed in heart, smooth muscle, neurons, pancreas, stomach, kidney	- generate background or leak K^+ conductance - myogenic in muscle - regulate excitability - ion and water transport - acid-base homeostasis - hormone secretion	**Highly specific, widespread and strong expression:** Muller glia IHC: TREK-1, -2 EP Astrocytes mRNA: TASK-1, -3, TREK-2 IHC: TASK-1, -2, -3 EP	 79 78 80, 81 82 81

$[K^+]_o$ - extracellular potassium concentration; pH_i - intracellular pH; pH_o - extracellular pH; PIP_2 - phosphatidylinositol-biphosphate; PKA - protein kinase A; PKC - protein kinase C; RMP - resting membrane potential

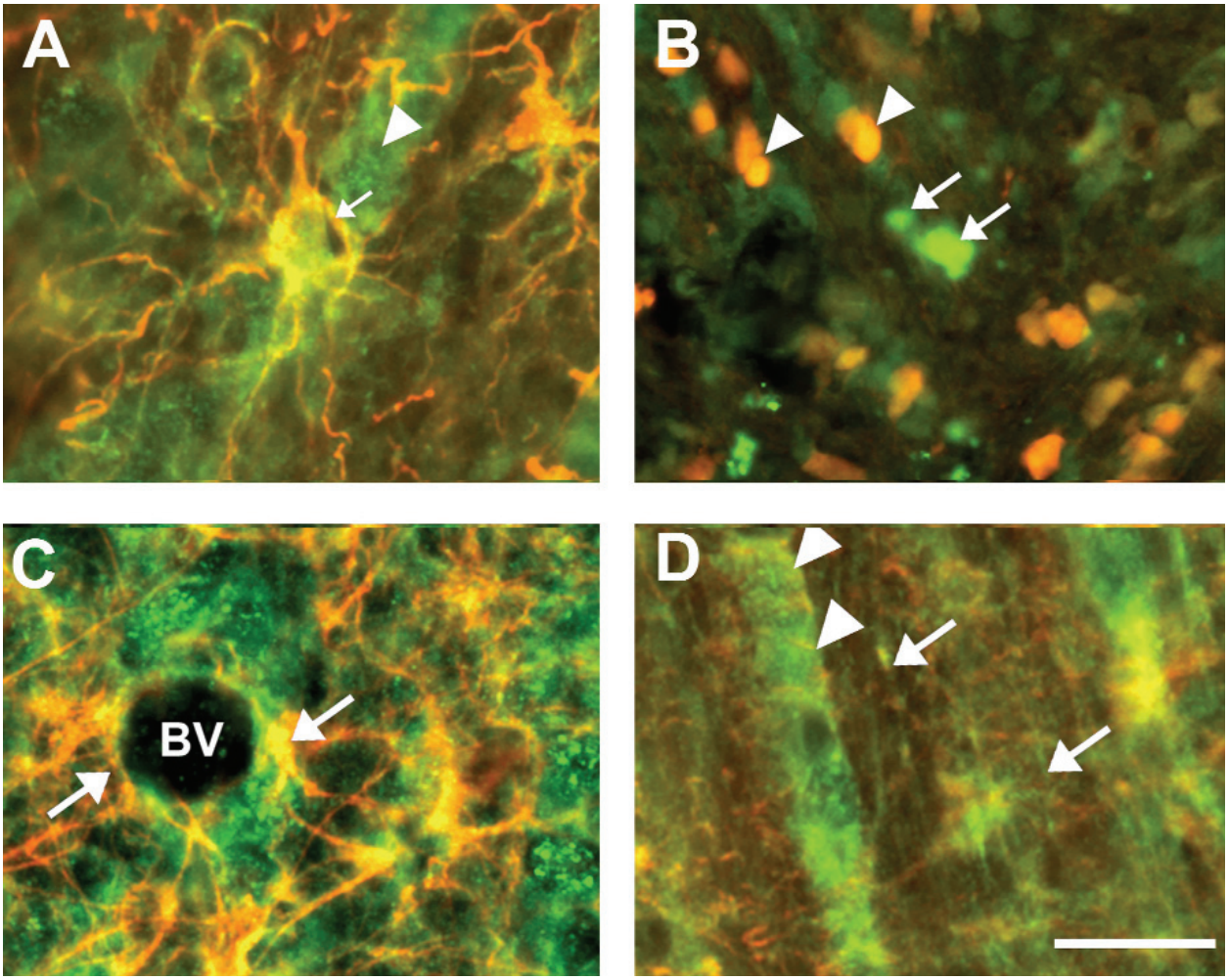

Fig. 1 Kir4.1 expression in white matter astrocytes and oligodendrocytes. Immunolabelling of sections of optic nerve with antibodies to Kir4.1 (green) and glial fibrillary acidic protein (GFAP) for astrocytes (red, A,C,D) or carbonic anhydrase II (CAII) for oligodendrocytes (red, B); co-expression appears yellow. (A) Kir4.1+/GFAP+ astrocytes (arrow) are sited amongst rows of Kir4.1+/GFAP- oligodendrocytes (arrowhead). (B) Kir4.1+/CAII+ oligodendrocytes (arrowheads) together with Kir4.1/CAII- cells (arrows), which are both astrocytes and CAII- oligodendrocytes. Kir4.1 immunolabelling in oligodendrocytes is strongest in cell bodies (B, arrows). (C) Strong Kir4.1 immunolabelling in astrocyte processes and perivascular end-feet (arrows) surrounding blood vessels (BV). (D) Between rows of interfascicular Kir4.1+/GFAP- oligodendrocytes (arrowheads), fine Kir4.1+/GFAP+ astrocyte processes (arrows) pass within the fascicles of myelinated axons to subserve nodes of Ranvier, the sites of action potential propagation and K^+ release. Bar = 25 µm in A, C, D and 40 µm in B. Adapted from Kalsi *et al.* [60] with permission.

and Kir2.3 [65]. Strong immunoreactivity for Kir2.1 and Kir2.3 has also been demonstrated for astrocytes *in vitro* [66, 67]. The expression of strongly rectifying Kir2.0 channels in glia suggests a prominent role for this channel in setting the glial RMP and in $[K^+]_o$ regulation. It has been suggested that functional Kir2.0 channels may be exclusively heteromeric, as has been indicated for Kir5.1 and the Kir3.0 subfamily [29–35]. Interestingly, heteromeric co-assembly of Kir4.1 with Kir2.1 results in a channel that is more strongly rectifying than homomeric Kir4.1 channels [32]. In contrast, co-assembly of Kir2.1 with Kir5.1 forms electrically silent channels [35], and co-assembly with Kir4.1 or Kir5.1 may respectively positively and negatively regulate Kir2.1 channels.

Comparison of immunolabelling for Kir2.0, Kir4.1, and Kir5.1 suggests co-assembly amongst these subtypes is a possibility in Bergmann glia, astrocytes and oligodendrocytes [52, 54, 59, 60, 64]. Similarly, the expression of Kir4.1 and Kir5.1, and Kir5.1 and Kir2.1 overlap in retinal Müller glia, but Kir2.1 and Kir4.1 are differentially expressed, suggesting these two channel subtypes have separate roles in these cells [51, 56, 58].

There is clear evidence that glia express ATP-sensitive Kir channels. These channels are composed of four Kir6.0 subunits and four sulphonylurea receptors (SUR1 or SUR2) and retinal Müller glia have been shown to express mRNA for Kir6.1 and Kir6.2 [63], and are immunopositive for Kir6.1 and SUR1 [68–70]. Astrocytes have been shown to express message for Kir6.2 and SUR1 in the dorsal vagal nucleus [71], and Kir6.2 mRNA and immunoreactivity has been demonstrated in astrocytes and oligodendrocytes in the corpus callosum and cerebellar white matter [72]. However, in other studies, no expression of Kir6.2 mRNA was found in astrocytes or oligodendrocytes [73], and immunoreactivity for only Kir6.1 and not Kir6.2 subunits was found in hippocampal, cortical and cerebellar astrocytes and cerebellar Bergmann glia [74]. The reasons for these discrepancies are unclear, but they may indicate heterogeneity of glial Kir6.0 channel expression, similar to that found for Kir2.0 and Kir4.1 channel subtypes (see above), and electrophysiological studies have shown heterogeneity in the expression of functional K_{ATP} channels in astrocytes in the dorsal vagal nucleus, cerebellum and hippocampus [71, 75, 76]. Due to the ATP-sensitivity of Kir6.0, they are only active when intracellular concentrations of ATP fall to very low levels and therefore serve to maintain the high K^+ conductance and hyperpolarized RMP in glia during metabolic challenge [68, 69, 76]. Furthermore, immunoreactivity for astroglial Kir6.1 in the cerebellum is localized to astrocyte processes surrounding synapses, and mirrors that of neuronal Kir6.2, suggesting glial and neuronal K_{ATP} channels may act in synergy during metabolic challenges in the brain [74].

The evidence for glial expression of G protein-activated Kir (GIRK) channels is sparse, which is perhaps surprising since they are regulated by neurotransmitters and might be expected to couple glial K^+ regulation with neuronal activity. Message for Kir3.1 and Kir3.2 has been found in Müller cells of the guinea-pig retina [63], although no immunostaining for Kir3.1, Kr3.2, Kir3.3 or Kir3.4 was observed in Müller cells of the frog retina [68]. Astrocytes have been shown to be immunopositive for Kir3.1 *in vitro* [67], and there is electrophysiological evidence of 5-HT and β-adgrenergic activated Kir with features of GIRK in cultured astrocytes and leech giant glia [77, 78]. The clearest evidence for glial GIRK is in cultured oligodendrocytes, in which activation of G protein-coupled receptors by 5-HT, somatostatin and ACh leads to rapid inhibition of GIRK [79, 80]. This is opposite to the effects of GIRK activation in heart muscle, and the function of these channels in oligodendrocytes is unknown.

The recently isolated family of tandem- or two-pore (2P) domain TREK and TASK K^+ channels are responsible for background or leak K^+ conductance in excitable tissues [42, 43]. Glial membranes are also characterised by a large K^+ conductance, which is a major determinant of their strongly negative RMP, and hence 2P-domain K^+ channels are of emerging interest in glia. Electrophysiological studies indicate that blockade of the TASK conductance depolarized Müller cell membranes by about 50% [81], and recent studies have shown that Müller glia of the amphibian retina are immunopositive for TREK-1 and TREK-2, but not TASK-1 or TASK-2 [82]. Message for TASK-1, TASK-3 and TREK-2 has been demonstrated by RT-PCR in cultured astrocytes [83, 84], and astrocytes have been shown to be immunopositive for TASK-1, TASK-2 and TASK-3 channels *in vivo* and in primary tissue culture [85]. Patch-clamp analysis indicated that astroglial TREK-2 channels are activated by pathophysiological increases in free arachidonic acid (AA), a characteristic of TREK channels, and regulate astrocytic swelling [84]. In Müller cells, TASK channels were depressed by intracellular acid pH, and activated by alkaline pH and cell swelling, but not by AA, and were characteristic of TASK channels [81]. Collectively, the results indicate that glial 2P-domain K^+ channels are involved in homeostasis of glial cell volume, and may have a protective role during metabolic disturbances and ischemia by maintaining a hyperpolarized RMP in conditions where Kir4.1 and Kir2 are blocked by intracellular acidification [81, 84].

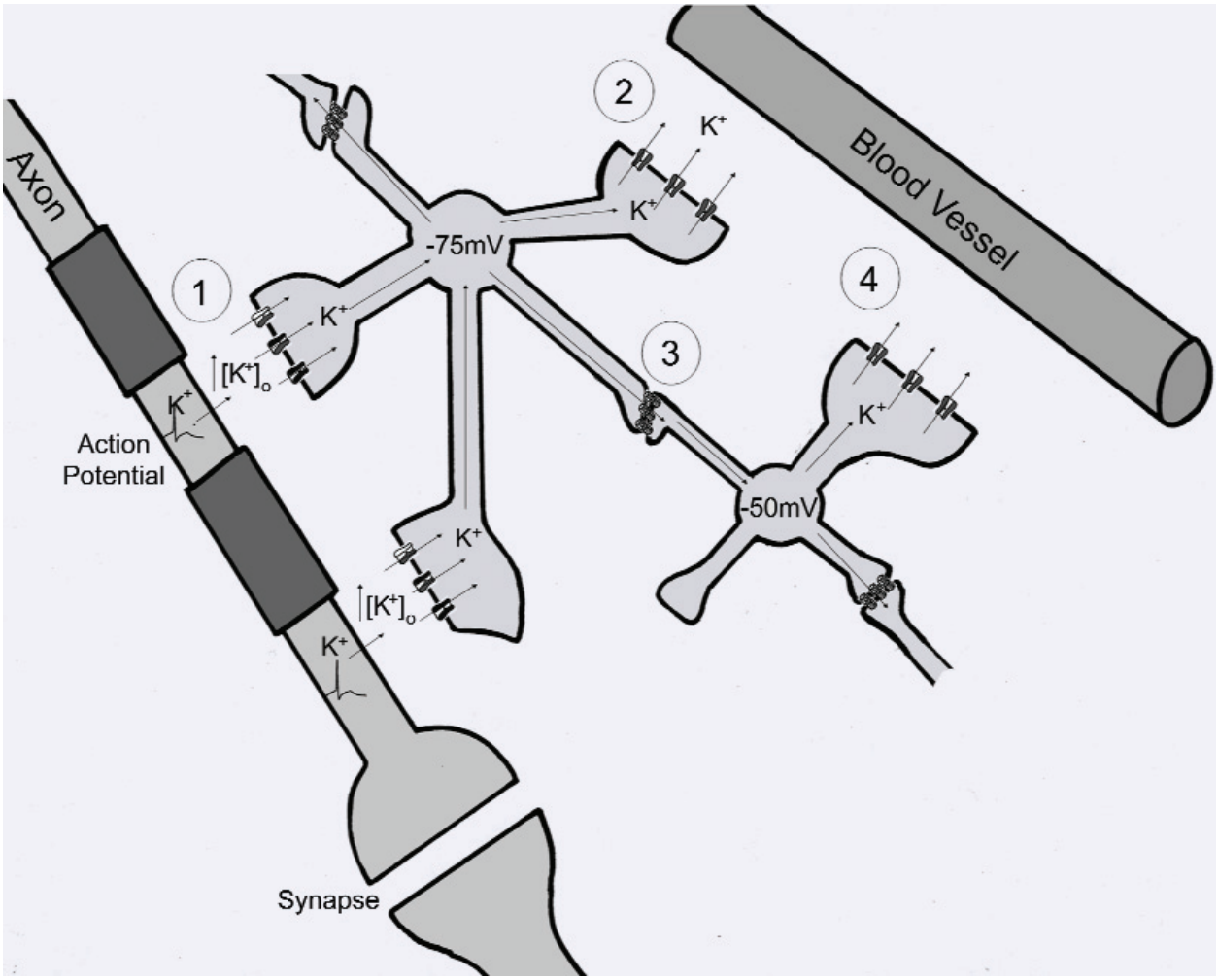

Fig. 2 A model of K^+ spatial buffering. Potassium released during action potential propagation is taken up by astrocytes processes at nodes of Ranvier and synapses *via* strongly rectifying Kir channels, both homomeric Kir2.1 channels and heteromeric Kir4.1/2.1 and Kir4.1/Kir5.1 channels (**1**). Potassium is extruded from glia at sites of low K^+ activity *via* weakly rectifying Kir4.1 homomeric channels (**2**). The astroglial syncytium contains a mixture of astrocytes with strongly negative and weakly negative RMPs. The strict dependence of Kir on both voltage and $[K^+]_o$ facilitates K^+ uptake into astrocytes with a strongly negative RMP at sites of high $[K^+]_o$ (**1**) and, following redistribution *via* gap junctions to astrocytes with weakly negative RMPs (3), facilitates the release of K^+ at sites of low $[K^+]_o$ (**4**).

Key roles for Kir4.1 in glial functions of K^+ regulation and myelination

The differences in conductance and sensitivity to intracellular and extracellular factors, including pH, ATP, G-proteins, neurotransmitters and hormones, suggests the different Kir channels expressed by glial cells may have multiple and distinct functional roles. Notwithstanding the diversity of glial Kir, a notable and consistent finding is the widespread and specific expression of Kir4.1 in CNS glia, strongly suggesting a special role for this K^+ channel in determining the physiological characteristics of glia. In Kir4.1 knock-out mice, ablation of Kir4.1 results in a dramatic reduction in Kir currents, depolarization and reduced capacity for K^+ buffering in glia [10, 15, 21]. Moreover, the subcellular localization of Kir4.1 channels is consistent with a specific role in the transport of K^+ by glia between neurons and blood vessels (Fig. 2). In astrocytes, the processes wrapping synapses and blood vessels are enriched with Kir4.1 and Kir5.1 channels [54, 59]. In retinal

Müller cells, the processes surrounding neurons express Kir4.1, Kir5.1 and Kir2.1 channels, whereas endfeet facing the vitreous and processes surrounding blood vessels are enriched with Kir4.1 channels [51, 56, 58]. This suggests a model in which K^+ released during neuronal activity is taken up by glia *via* strongly rectifying heteromeric Kir4.1/Kir5.1 and Kir4.1/2.1 channels, and possibly homomeric Kir2.1 channels, and K^+ is then extruded from glia into extracellular 'sinks' *via* weakly rectifying Kir4.1 homomeric channels (Fig. 2). Notably, astrocytes are physiologically heterogeneous and the textbook view of astrocytes with uniformly strongly negative RMPs close to the E_K is an over-simplification and astrocytes *in situ* display both weakly and strongly negative RMPs [12–15, 21]. The strict dependence of Kir on voltage and $[K^+]_o$ allows K^+ uptake into hyperpolarised cells when $[K^+]_o$ is raised, but K^+ release when the cell is depolarised and $[K^+]_o$ is low. Accordingly, K^+ released during neuronal activity will be rapidly taken up by astrocytes with a strongly negative RMP and redistributed *via* gap junctional cell-cell coupling to astrocytes with weakly negative RMPs, which will tend to release K^+ at sites of low K^+ activity, such as blood vessels. Potassium extrusion is not only at sinks, and glia play a major role in the post-stimulus recovery of $[K^+]_o$, whereby K^+ released during neuronal activity is taken up primarily via the activity of Na^+-K^+ pumps, and is returned to the neuronal extracellular milieu by K^+ efflux through glial Kir channels at processes surrounding synapses and nodes of Ranvier [86]. Furthermore, the astrocyte RMP and Kir expression are physiologically dynamic and are regulated by diverse extracellular and intracellular factors that provide mechanisms for coupling glial function with neuronal activity (Table 1), including extracellular glutamate, intracellular cyclic AMP (cAMP) and protein kinase A (PKA) levels, extracellular and intracellular ATP, extracellular and intracellular pH, and the extent of cell-cell coupling *via* gap junctions [12, 13, 15, 21, 24, 26].

It should not be neglected that oligodendrocytes are strongly immunopositive for Kir4.1 and their paranodal cytoplasmic loops are ideally sited for the regulation of K^+ released during action potential propagation [52, 60]. This possibility is supported by the correlation between the development of $[K^+]_o$ regulation, the developmental increase in oligodendroglial Kir currents and Kir4.1 expression, and a developmental negative shift in the RMP [19–21, 60, 87]. Significantly, the developmental shift in Kir currents and RMP in oligodendrocytes are lost in Kir4.1 knock-out mice, and the most dramatic consequence was a block of oligodendrocyte maturation and severe hypomyelination [20, 61, 62]. These studies indicate a critical role for Kir4.1 in generating the negative oligodendroglial RMP and the formation and maintenance of myelin.

Overview

The diverse range of Kir expressed by glia maintain the characteristic high K^+ selectivity of their cell membranes and strongly negative RMPs under diverse physiological and pathophysiological conditions. There is substantial evidence that the Kir4.1 channel subtype is selectively expressed by glia in the CNS and has specific and special roles in glia. Studies in knock-out mice demonstrate that Kir4.1 channels are essential for the 'textbook' physiological properties of glia and for the primary glial functions of K^+ buffering and myelination.

Acknowledgements

Our work on Kir4.1 was supported by a grant from the BBSRC

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