Chapter 6
Regulation of Potassium by Glial Cells in the Central Nervous System

Paulo Kofuji and Eric A. Newman

Contents

Rapid changes in extracellular K^+ concentration ($[K^+]_o$) in the mammalian central nervous system (CNS) are counteracted by simple passive diffusion as well as by cellular mechanisms of K^+ clearance. Regulation of $[K^+]_o$ can occur via glial or neuronal uptake of K^+ ions through transporters or K^+-selective ion channels. The best studied mechanism of $[K^+]_o$ regulation in the brain is *K^+spatial buffering*, wherein the glial syncytium disperses local extracellular K^+ increases by transferring K^+ from sites of elevated $[K^+]_o$ to those with lower $[K^+]_o$. In recent years, K^+ spatial buffering has been implicated or directly demonstrated by a variety of experimental approaches, including electrophysiological and optical methods. A specialized form of spatial buffering termed *K^+siphoning* takes place in the vertebrate retina, where glial Müller cells express inwardly rectifying K^+ channels (Kir channels) positioned in membrane domains near to the vitreous humor and blood

E.A. Newman
Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA
ean@umn.edu

V. Parpura and P.G. Haydon (eds.), *Astrocytes in (Patho)Physiology of the Nervous System*,
DOI: 10.1007/978-0-387-79492-1_6, © Springer Science+Business Media, LLC 2009

vessels. This highly compartmentalized distribution of Kir channels in retinal glia directs K^+ ions from the synaptic layers to the vitreous humor and blood vessels. Here, we review the principal mechanisms of $[K^+]_o$ regulation in the CNS and recent molecular studies on the structure and function of glial Kir channels. We also discuss intriguing new data that suggest a close physical and functional relationship between Kir and water channels in glial cells.

6.1 Potassium in the Extracellular Space of the Central Nervous System

Neurons are bathed in extracellular fluid that has a high concentration of Na^+ ions and a low concentration of K^+ ions. The relative concentrations of these cations inside of cells are reversed. The resulting ionic gradients across the cell membrane are crucial to the generation of essential neuronal signals, including the resting membrane potential, the action potential, and synaptic potentials. Because of the low baseline concentration of extracellular K^+($[K^+]_o$), and to the limited volume of extracellular space, even modest efflux of K^+ from neurons can elicit considerable changes in $[K^+]_o$ (Nicholson and Sykova, 1998; Kume-Kick et al., 2002). These $[K^+]_o$ changes can influence a wide variety of neuronal processes, including the maintenance of the resting membrane potential, activation, and inactivation of voltage gated ion channels, the efficacy of synaptic transmission, and electrogenic transport of neurotransmitters. Thus, it is not surprising that the CNS possesses robust cellular mechanisms to regulate $[K^+]_o$. Under normal physiological conditions, these mechanisms maintain $[K^+]_o$ close to 3 mM. When K^+ regulatory mechanisms are overwhelmed under pathophysiological conditions such as spreading depression and ischemia, extracellular $[K^+]_o$ can reach values as high as 60 mM or more (Somjen, 2001, 2002). These extreme $[K^+]_o$ levels severely depolarize neurons, rendering them inactive.

Normal neuronal activity in the CNS results in modest variations in $[K^+]_o$. Light stimulation in the cat produces slow, transient $[K^+]_o$ increases, smaller than 1 mM, in the primary visual cortex (Fig. 6.1a) (Singer and Lux, 1975; Connors et al., 1979). Similarly, light stimulation in the frog and cat induces $[K^+]_o$ increases of less than 1 mM in the inner and outer plexiform layers of the retina and $[K^+]_o$ decreases in the outer retina and subretinal space (Fig. 6.1b) (Karwoski et al., 1985; Frishman et al., 1992). In cat spinal cord, rhythmic flexion/extension of the knee joint produces $[K^+]_o$ increases of 1.7 mM (Heinemann et al., 1990). Significantly higher $[K^+]_o$ elevations are evoked by direct electrical stimulation of afferent pathways and by induction of seizure activity. Even under intense, high-frequency stimulation, however, $[K^+]_o$ does not exceed a plateau or ceiling level of 10–12 mM. This ceiling level is seen in the cat somatosensory cortex (Heinemann and Lux, 1977), in the cat thalamus (Gutnick et al., 1979), and in the rat optic nerve (Connors et al., 1982; Ransom et al., 1986). The ceiling level is exceeded only under pathophysiological conditions such as anoxia (Vyskocil et al., 1972) or spreading depression (Somjen, 2002).

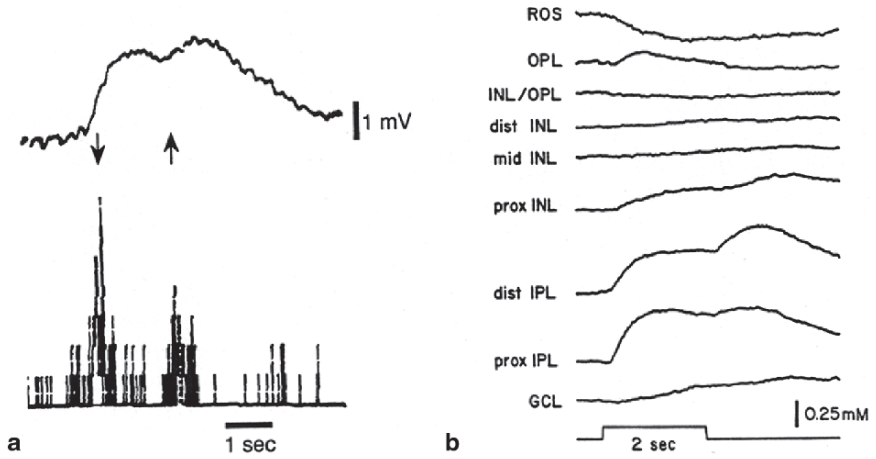

Fig. 6.1 Activity-evoked changes in $[K^+]_o$ in the cat striate cortex and frog retina. (**a**) Upper trace. Dynamic $[K^+]_o$ changes in the cat striate cortex evoked by stimulation of the receptive field of hypercomplex cells. $[K^+]_o$ changes were measured with a double-barreled K^+-sensitive microelectrode. *Arrows* represent bars of light moving down or up in a cell s receptive field. The 1-mV scale bar corresponds to ~0.17 mM. *Lower trace*. Spike activity recorded in the reference barrel of the K^+-sensitive microelectrode. [From Singer and Lux (1975), with permission.] (**b**) $[K^+]_o$ changes in different layers of the frog retina recorded with a K^+-sensitive microelectrode. A 2-s light stimulus evokes $[K^+]_o$ increases in the inner plexiform layer (IPL) and outer plexiform layer (OPL) and a $[K^+]_o$ decrease in the subretinal space (ROS). [From Karwoski et al. (1985), with permission.]

The careful control of $[K^+]_o$ within the brain is due to efficient K^+ regulatory mechanisms that operate in the CNS. Neuronal depolarization is accompanied by an efflux of K^+ into extracellular space. Even modest neuronal activity results in significant $[K^+]_o$ increases. Potassium efflux due to a single action potential can raise $[K^+]_o$ by 25% (Ransom and Sontheimer, 1992). Potassium regulatory mechanisms are responsible for maintaining $[K^+]_o$ near 3 mM during normal brain activity and prevent $[K^+]_o$ from exceeding 10–12 mM, even during tetanic stimulation or during seizure activity. This chapter reviews glial mechanisms that contribute to $[K^+]_o$ regulation in the CNS. The chapter is an updated version of a previous review (Kofuji and Newman, 2004).

6.2 Overview of K^+ Regulatory Mechanisms

Potassium regulation in the CNS is mediated by two types of mechanisms: net K^+ uptake and K^+ spatial buffering (Fig. 6.2) (Newman, 1995; Amedee et al., 1997; Somjen, 2002). For K^+ uptake, excess extracellular K^+ is temporarily taken up and sequestered within glial cells. (In theory, excess K^+ could also be sequestered within quiescent neurons. However, neuronal uptake is not thought to play

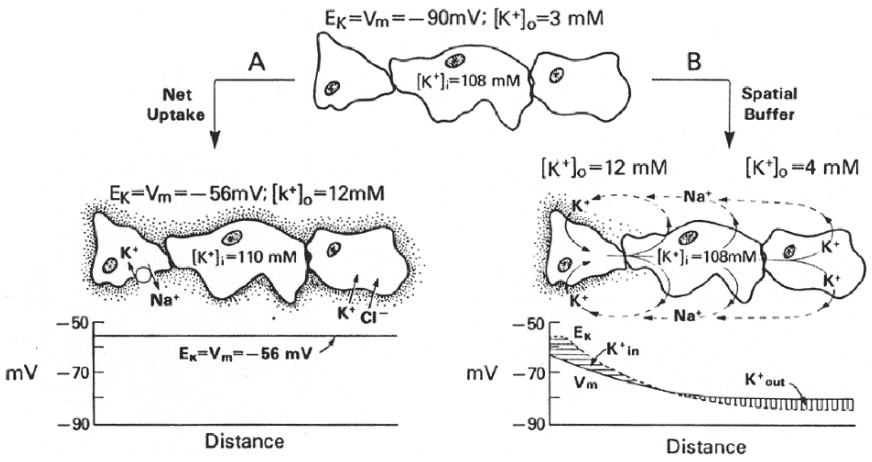

Fig. 6.2 Diagram depicting the role of glial cells in $[K^+]_o$ regulation. *Top*: Glial cells are electrically coupled via gap junctions forming a functional syncytium. With $[K^+]_o$ equaling 3 mM, the glial syncytium has a membrane potential of -90 mV. (**a**) Net K^+ uptake mechanism. When $[K^+]_o$ is increased, glial cells accumulate K^+ either by the activity of the Na^+, K^+-ATPase or by a pathway in which K^+ is cotransported with Cl^-. In this mechanism of K^+ regulation, the membrane potential of the glial syncytium equals −56 mV and is spatially uniform. (**b**) Potassium spatial buffering mechanism. Local increases in $[K^+]_o$ produce a glial depolarization that spreads passively through the glial syncytium. The local difference between the glial syncytium membrane potential (V_m) and the K^+ equilibrium potential (E_K) drives K^+ influx in regions of elevated $[K^+]_o$ and K^+ efflux in distant regions. Intracellular currents are carried primarily by K^+ and extracellular currents by Na^+ and Cl^-. [From Orkand (1986), with permission.]

a significant role in the rapid removal of K^+ from extracellular space and will not be considered here.) To preserve electroneutrality, K^+ influx into glial cells is accompanied by either influx of anions such as Cl^- or by efflux of cations such as Na^+ (Fig. 6.2a). Net K^+ uptake can occur by an active process, by the action of the Na^+, K^+-ATPase (Na^+ pump), or passively, by K^+ flux through transporters or K^+ channels. When neuronal activity decreases and $[K^+]_o$ falls to near baseline levels, the K^+ sequestered within glial cells is released and is returned to the neurons by the action of neuronal Na^+ pumps. An influx of water accompanies net K^+ uptake into glia, resulting in glial-cell swelling (Dietzel et al., 1980).

Potassium regulation in the CNS can also be mediated by K^+ spatial buffering. In this process, K^+ is transferred from regions of elevated $[K^+]_o$ to regions of lower $[K^+]_o$ by a current flow through glial cells (Orkand et al., 1966). The K^+ current is driven by the difference between the glial syncytium membrane potential (V_m) and the local K^+ equilibrium potential (E_K). In regions of increased $[K^+]_o$, there is a net driving force causing K^+ to flow into the glial cells (Fig. 6.2b). This K^+ entry generates a local depolarization, which propagates electrotonically through individual glial cells and through the glial-cell syncytium. As a result, there is a net driving force causing K^+ to flow out of the glial cells in regions where $[K^+]_o$ is low. The

redistribution of K^+ by the spatial buffering mechanism reduces local $[K^+]_o$ increases with little net gain of K^+ within the glial cells. The overall efficiency of the spatial buffer process will depend, in part, on the electrical space constant of the glial-cell syncytium (Newman, 1995). In certain CNS regions close to fluid reservoirs, such as the retina, the K^+ spatial buffer mechanism can efficiently redistribute K^+ by a current flow within single glial cells rather than through a network of cells. In these cases, K^+ influx occurs in one region of the glial cell and efflux occurs through another cell region, typically the endfoot process. This specialized form of spatial buffering is termed *K^+siphoning*.

6.3 Net Uptake of K^+

Net K^+ uptake is mediated by active uptake via the Na^+ pump and by passive uptake, mediated by Na^+–K^+–Cl^- cotransporters and by K^+ and Cl^- channels. The Na^+ pump plays a principal role in K^+ regulation. The Na^+ pump is a transmembrane enzyme that functions as an electrogenic ion transporter in all cells (Kaplan, 2002; Jorgensen et al., 2003). With each cycle of the Na^+ pump, three Na^+ are expelled and two K^+ are moved into the cell, and one ATP molecule is hydrolyzed. The Na^+ pump is activated by intracellular Na^+ and extracellular K^+. Local $[K^+]_o$ increases generated by increased neuronal activity will result in raised pump activity and to increased influx of K^+ (assuming that the extracellular K^+ site of the pump is not saturated and the intracellular Na^+ concentration is not limiting (Sweadner, 1995)). Different Na^+ pump isoforms have varying affinities for K^+ at the extracellular K^+ site. The Na^+ pump expressed in glial cells is better suited for regulating $[K^+]_o$ than is the neuronal isoform in that the glial isoform has a lower affinity for extracellular K^+(Franck et al., 1983; Reichenbach et al., 1992). In retina, for example, the principal glial cell, the Müller cell, expresses a Na^+ pump isoform that is maximally activated at 10–15 mM of $[K^+]_o$ while the isoform present in rod photoreceptors saturates at $[K^+]_o$ as low as 3 mM (Reichenbach et al., 1992). If the glial isoform of the Na^+ pump is saturated at 3 mM of $[K^+]_o$, then increases in $[K^+]_o$ above this level would not increase pump activity and the pump could not contribute to $[K^+]_o$regulation.

Reports from several laboratories demonstrate that the Na^+ pump contributes to K^+ regulation in the CNS. Electrical stimulation in guinea pig cortical slices results in a transient accumulation of K^+ ions and in a simultaneous depletion of Na^+ ions within glial cells (Ballanyi et al., 1987). A substantial fraction of this K^+ accumulation is prevented by pharmacological blockade of the Na^+ pump (Ballanyi et al., 1987). Similarly, in hippocampal slices, blockade of the Na^+ pump increases baseline $[K^+]_o$ and prevents the rapid clearance of K^+ following neuronal stimulation (Fig. 6.3) (D'Ambrosio et al., 2002). In the rat optic nerve, clearance of K^+ accumulation following axonal stimulation is highly temperature dependent ($Q_{10} = 2.6$), as expected for a carrier-mediated process, and is largely blocked by Na, K-ATPase inhibitors (Ransom et al., 2000).

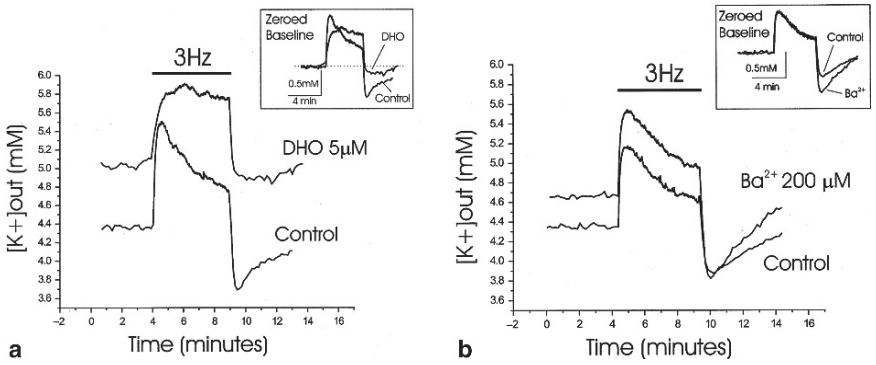

Fig. 6.3 Differential roles for the Na^+, K^+-ATPase and Kir channels in K^+ regulation in rat hippocampal slice. (**a**) In control condition, 3-Hz antidromic stimulation induces a $[K^+]_o$ increase in area CA3 that peaks at 5.5 mM followed by a decline to 4.7 mM. With the addition of the sodium pump inhibitor dihydroouabain (DHO), the baseline $[K^+]_o$ increases to 5.1 mM. Antidromic stimulation (3 Hz) induces a $[K^+]_o$ increase to 5.9 mM but there is no $[K^+]_o$ recovery phase. Also absent is the undershoot of $[K^+]_o$ following the stimulation period. In the inset, the two traces are shown superimposed with the baselines zeroed. (**b**) In control condition, 3-Hz antidromic stimulation induces a $[K^+]_o$ increase that peaks at 5.2 mM followed by an undershoot in $[K^+]_o$. With the addition of Ba^{2+}, baseline $[K^+]_o$ increases and the undershoot in $[K^+]_o$ following stimulation is more pronounced. In the *inset*, the two traces are shown superimposed and the baselines zeroed. [From D'Ambrosio et al. (2002), with permission.]

Na^+–K^+–Cl^- cotransporters also play an important role in the regulation of $[K^+]_o$ in the CNS. These transporters are integral membrane proteins that transport Na^+, K^+, and Cl^- ions into and out of cells in an electrically neutral manner, often with a stoichiometry of $1Na^+$:$1K^+$:$2Cl^-$ (Haas and Forbush, 1998). Two Na^+–K^+–Cl^- cotransporter isoforms have been identified: NKCC1, which is present in a wide variety of secretory epithelia and nonepithelial cells; and NKCC2, which is present exclusively in the kidney (Haas and Forbush, 1998). Both NKCC isoforms are members of a diverse family of cation-chloride cotransport proteins that share a common predicted membrane topology and are sensitive to loop diuretics such as bumetanide and furosemide (Haas and Forbush, 1998). In cultured astrocytes, intracellular accumulation of K^+ following an increase in $[K^+]_o$ can be partially blocked by furosemide or bumetanide or by removal of external Na^+ and Cl^- (Kimelberg and Frangakis, 1985; Walz, 1992; Rose and Ransom, 1996). More recently, the role of Na^+–K^+–Cl^- cotransporters in $[K^+]_o$ homeostasis has also been demonstrated by optical methods. In the rat optic nerve, intrinsic optical signals reveal that an increase in $[K^+]_o$ induces astrocyte swelling that is reversibly depressed by furosemide and bumetanide (MacVicar et al., 2002). A monoclonal antibody to the NKCC1 isoform of the Na^+–K^+–Cl^- cotransporter shows that the transporter is expressed in astrocytes from the optic nerve (MacVicar et al., 2002), suggesting the involvement of this particular Na^+–K^+–Cl^- cotransporter isoform in K^+ regulation.

6.4 Potassium Spatial Buffering

Two conditions are necessary for efficient K^+ spatial buffering as originally proposed by Orkand et al. (1966): (1) the glial cells should form a syncytium in which K^+ currents can traverse relatively long distances; and (2) these cells should be highly and selectively permeable to K^+, which both enters and exits through the glial-cell membranes. As described below in Sect. 6.6 on K^+ siphoning in retina, spatial buffer currents can efficiently dissipate $[K^+]_o$ increases by flowing through single cells as well as through a syncytium of coupled glial cells. Several lines of evidence demonstrate that astrocytes do indeed form a functional syncytium that allows intercellular diffusion of ions and other signaling molecules (Nagy and Rash, 2000; Rouach et al., 2002). Such extensive cellular coupling is due to the high density of gap junctional channels (connexins, Cx) in glial cells (Dermietzel, 1998; Rouach et al., 2002). Immunocytochemical and in situ hybridization studies reveal that astrocytes express multiple connexins, including Cx30, Cx40, Cx43, and Cx45 (Dermietzel, 1998; Dermietzel et al., 2000; Zahs et al., 2003). Among these, Cx43 and Cx30 seem to be the most important for coupling, which is significantly reduced in astrocytes of Cx43 knockout (KO) and Cx43/Cx30 double KO mice (Dermietzel et al., 2000; Wallraff et al., 2006).

Recent studies have demonstrated that there are two classes of CNS glia that resemble astrocytes: "passive astrocytes," also termed GluT, that have ohmic current–voltage relations and express glutamate transporters and"complex glia," also termed GluR, that have rectifying current–voltage relations and express ionotropic glutamate receptors but not transporters (Matthias et al., 2003; Zhou et al., 2006). Passive astrocytes express connexins that are coupled to each other and presumably participate in K^+ spatial buffering. Complex glia do not express connexins, are not coupled together, and presumable do not conduct spatial buffer currents.

Numerous studies have shown that glial-cell membranes are highly and almost exclusively permeable to K^+ (Sontheimer, 1994). The principal K^+ channels found in glial cells are the inwardly rectifying K^+ (Kir) channels, which allow K^+ ions to flow more readily in the inward than outward direction (Doupnik et al., 1995; Stanfield et al., 2002). These channels have a high open probability at the normal resting membrane potential and thus allow both glial K^+ influx and efflux. Kir channels in glia have been described in many CNS regions, including mammalian astrocytes from the optic nerve (Barres et al., 1990), spinal cord (Ransom and Sontheimer, 1995), and other brain regions (Sontheimer, 1994). Although glia may express additional types of K^+ channels, such as Ca^{2+}- and voltage-dependent K^+ channels (Sontheimer, 1994), these other channel types are largely inactive at the hyperpolarized glial resting membrane potential (−60 to −90 mV) (Kuffler et al., 1966; Dennis and Gerschenfeld, 1969). Astrocytes may also express two-pore domain K^+ channels (see below). An important biophysical property of Kir channels is that their slope conductance increases with elevations in $[K^+]_o$ by a square root relation (Stanfield et al., 2002). This unique property of Kir channels allows K^+ conductance increases in glial cells, and therefore, enhanced K^+ clearance rates, when $[K^+]_o$ is raised (Newman, 1993).

An important implicit assumption for the K^+ spatial buffering mechanism is the low permeability of glial cells to anions such as Cl^-, as the low anion conductance ensures that the net uptake of KCl will not occur when $[K^+]_o$ increases. Unfortunately, there is no consensus concerning glial-cell Cl^- permeability in native tissue (Walz, 2002). Although a relatively high basal Cl^- conductance has been reported in glial cells of guinea-pig olfactory cortex (Ballanyi et al., 1987), other studies have failed to demonstrate a significant Cl^- conductance for glial cells in situ (Walz, 2002).

6.5 Evidence for K^+ Spatial Buffering

Orkand et al. (1966) reported that, in amphibians, stimulation of the optic nerve leads to slow depolarization and repolarization of the glial cells surrounding non-myelinated axons. These slow glial membrane potential changes were thought to reflect K^+ transfer by glial cells via the K^+ spatial buffering mechanism. Subsequently, support for the K^+ spatial buffering hypothesis came from measurements of extracellular field potentials. The transcellular transfer of K^+ ions from areas of elevated $[K^+]_o$ to lower $[K^+]_o$ generates return current loops in the extracellular space, giving rise to extracellular field potentials. These activity-induced slow extracellular field potentials are generated in various CNS regions, including the cortex and retina (Gardner-Medwin et al., 1981; Dietzel et al., 1989). In the retina, slow extracellular field potentials (slow PIII and M waves) are generated upon light stimulation (Xu and Karwoski, 1997; Karwoski and Xu, 1999). Current source density analysis indicates that these waves originate from K^+ spatial buffering by retinal glial cells (Xu and Karwoski, 1997; Karwoski and Xu, 1999). The transfer of K^+ ions by retinal glial cells, which generates the slow PIII wave, buffers the photoreceptor-based light-evoked decrease in $[K^+]_o$ in the outer retina. As expected, these K^+ spatial buffering fluxes are abolished by blocking retinal glial-cell K^+ channels with Ba^{2+} (Oakley et al., 1992; Kofuji et al., 2000).

Measurements of activity-dependent $[K^+]_o$ changes in the cortex and cerebellum show that $[K^+]_o$ varies with depth and time in a manner consistent with transcellular transfer of K^+ ions (Gardner-Medwin and Nicholson, 1983). These $[K^+]_o$ changes were not abolished by Na^+ pump inhibitors, indicating that they are likely due to a passive K^+ transport mechanism such as K^+ spatial buffering (Gardner-Medwin and Nicholson, 1983). Similar results were obtained in the drone retina, where it was estimated that about 10 times more K^+ ions move as a result of spatial buffering than by simple diffusion through the extracellular space (Coles et al., 1986).

More direct evidence for K^+ spatial buffering has been provided by optical imaging of brain slices (Holthoff and Witte, 2000). When K^+ ions are transferred via glial cells, there is a shrinkage of the extracellular space in areas of K^+ influx and swelling in areas of K^+ efflux (Dietzel et al., 1980). Changes in extracellular volume following neuronal stimulation can be demonstrated by monitoring intrinsic optic signals (IOS) in brain slices. As predicted for K^+ spatial buffering, stimulation of cortical areas promoted shrinkage of the extracellular space in the

stimulated region followed by swelling in the layers above and below the stimulated area (Fig. 6.4) (Holthoff and Witte, 2000). This swelling in the extracellular space was associated with local increases in $[K^+]_o$ and was dependent on gap junctional coupling (Holthoff and Witte, 2000). These gap junctionally-dependent changes in extracellular space are consistent with K^+ spatial buffering in the mammalian cortex.

Recent studies have directly evaluated the importance of K^+ spatial buffering to the regulation of $[K^+]_o$ in the brain. Activity-dependent increases in $[K^+]_o$ have been

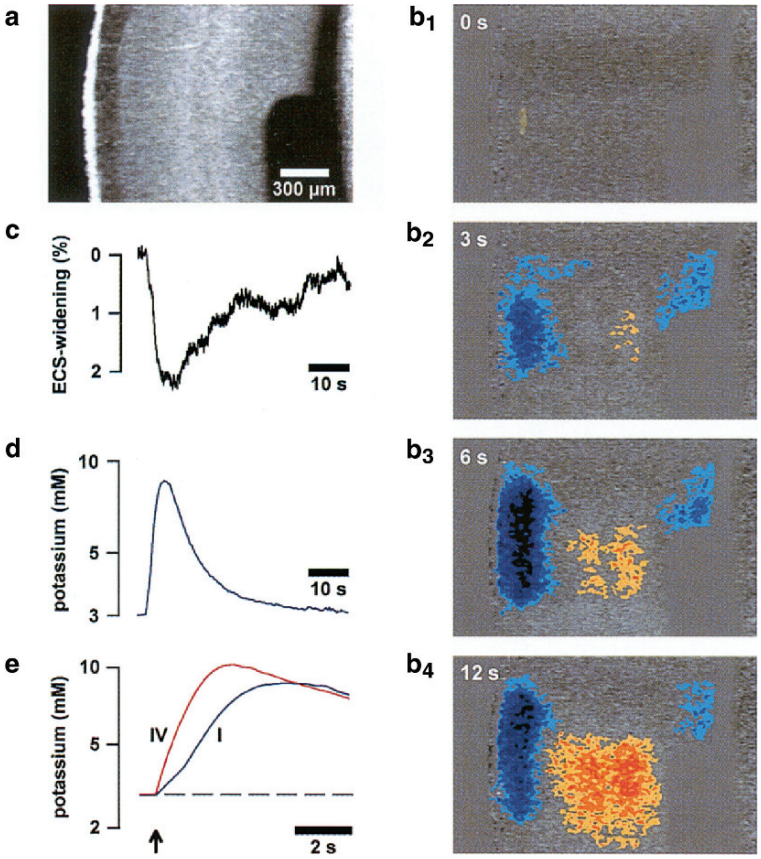

Fig. 6.4 Potassium spatial buffering in the rat cortex. (**a**) Image of a brain slice viewed with darkfield optics. (**b**1–4) Time course of intrinsic optical signal (IOS) changes upon neuronal stimulation in middle cortical layers. *Red colors* represent IOS increases while *blue colors* represent IOS decreases. These correspond to shrinking and widening of the extracellular space, respectively. Note that extracellular space shrinks in the middle cortical layers and widens in the most superficial and deep cortical layers, as predicted for the K^+ spatial buffering mechanism. (**c**) Time course of extracellular space widening in cortical layer I, measured independently, confirming the IOS results. (**d**) Time course of $[K^+]_o$ increase in layer I. (**e**) Time course of $[K^+]_o$ increase in layer I (*blue*) and in layer IV (*red*). [From Holthoff and Witte (2000), with permission]. (*See Color Plates*).

measured in brain slices of wild-type mice and in transgenic mice lacking astrocyte connexin and K^+-channel expression. If K^+ spatial buffering plays an important role in regulating $[K^+]_o$; then $[K^+]_o$ regulation should be compromised in the transgenic animals. In hippocampal slices of transgenic mice lacking Cx30 and Cx43 connexins, astrocytes were completely uncoupled (Wallraff et al., 2006). In these animals, activity-dependent $[K^+]_o$ increases were larger and clearance of the increases was slower. However, changes in $[K^+]_o$ regulation were modest in the transgenic animals, demonstrating that K^+ spatial buffering though the glial syncytium is not the only mechanism contributing to $[K^+]_o$ regulation. Similar conclusions were reached in a study of $[K^+]_o$ regulation in the brain stem in transgenic mice lacking Kir4.1 K^+ channels, the principal K^+ channel of astrocytes (Neusch et al., 2006). In transgenic animals, clearance of $[K^+]_o$ increases was slowed and the $[K^+]_o$ undershoot, which follows $[K^+]_o$ increases, was larger. However, rhythmic bursting activity of brain stem neurons was not altered in the Kir4.1 KO animals.

6.6 Potassium Siphoning

Regulation of $[K^+]_o$ by K^+ spatial buffering posits that K^+ is redistributed from regions of high $[K^+]_o$ to regions where $[K^+]_o$ is lower by a current flow through a network of electrically coupled glial cells. However, a redistribution of extracellular K^+ could also occur via a current flow through single glial cells, particularly, if these cells are elongated. This is the case for Müller cells, the principal glial cell of the retina (Newman and Reichenbach, 1996). Müller cells display morphological polarization with an endfoot process in close apposition to the vitreous and apical microvilli projecting into the subretinal space (Newman and Reichenbach, 1996). The membrane of Müller cells has a high K^+ conductance and is selectively permeable to K^+ (Newman, 1985). The high K^+ conductance of these cells is due to the abundant expression of Kir4.1 inwardly rectifying K^+ channels (Newman, 1993; Kofuji et al., 2000).

Kir4.1 channels are unevenly distributed along the membrane of Müller cells. Potassium-channel distribution has been mapped by monitoring cell responses to focal increases in extracellular K^+ concentration (Newman, 1984). In amphibian Müller cells, K^+ channels are highly concentrated in the endfoot process, with 94% of the total K^+ conductance localized to this relatively small subcellular domain (Newman, 1984; Brew et al., 1986).

The observation of a highly nonuniform distribution of Kir channels in Müller cells led to the hypothesis that excess K^+ released from retinal neurons is selectively directed, or "siphoned," to the vitreous humor (Newman et al., 1984; Newman, 1987a). This hypothesis, termed *K^+siphoning*, is a specialized form of the spatial buffering mechanism in which the nonuniform distribution of K^+ channels in glial cells directs excess K^+ into large reservoirs such as the vitreous humor (Fig. 6.5). In mammalian retinas, high densities of Kir channels are found on Müller cell endfeet contacting blood vessels, as well as on vitreal endfeet (Newman, 1987b, 1993;

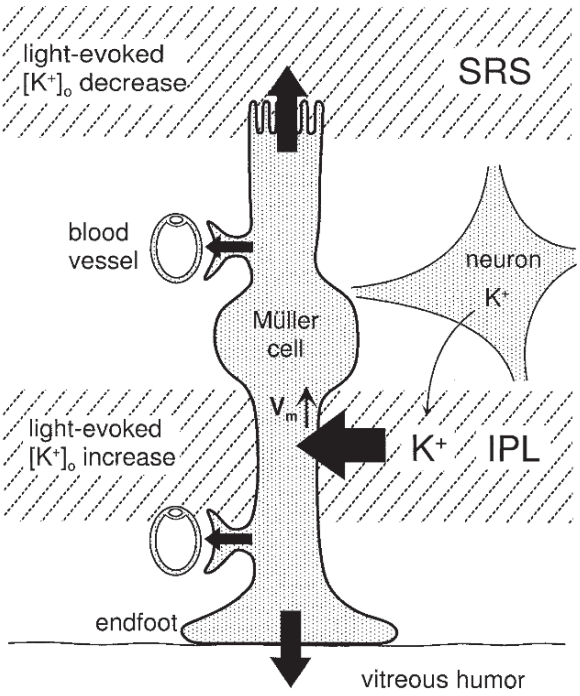

Fig. 6.5 Potassium siphoning in the retina. Potassium released from active neurons in the inner plexiform layer (IPL) generates a $[K^+]_o$ increase and an influx of K^+ into Müller cells, the principal glial cells of the retina. Potassium influx depolarizes the Müller cell and induces an efflux of an equal amount of K^+ from other cell regions. Potassium efflux occurs preferentially from Müller cell endfeet, where K^+-channel density is maximal, both at the vitreous humor and at processes enveloping blood vessels. Potassium efflux also occurs from the Müller-cell apical processes in the subretinal space (SRS), where light stimulation evokes a $[K^+]_o$ decrease. [From Newman (1996b), with permission.]

Kofuji et al., 2000). In these species, K^+ will be siphoned onto the blood vessels as well as into the vitreous.

Potassium siphoning contributes significantly to $[K^+]_o$ regulation in the retina. In the amphibian retina, light stimulation evokes rapid $[K^+]_o$ increases in the synaptic layers (Fig. 6.1b; inner plexiform layer (IPL) and outer plexiform layer (OPL)) and a slower increase in the vitreous humor (Fig. 6.1b; GCL). When Müller cell K^+ siphoning is interrupted by Ba^{2+} block of Kir channels, light-evoked $[K^+]_o$ increases within the retina grow larger, clearance of the $[K^+]_o$ increases is slowed, and the K^+ increase in the vitreous humor is reduced (Karwoski et al., 1989). These results demonstrate that Müller cells transfer excess K^+ from the retina to the vitreous by a K^+ siphoning current. Similarly, in the cat retina, light-evoked $[K^+]_o$ increases in the inner plexiform layer are threefold larger following Ba^{2+} block of Kir channels (Frishman et al., 1992), confirming that glial-mediated K^+ siphoning currents play an important role in limiting large variations in $[K^+]_o$.

Potassium siphoning may contribute to $[K^+]_o$ regulation in the brain as well. In hippocampal slices, clearance of K^+ released from pyramidal cells is dependent on glial Kir channels, as $[K^+]_o$ regulation is compromised by Ba^{2+} block of the channels. However, $[K^+]_o$ clearance within the stratum radiatum is not reduced in Cx43/Cx30 double KO animals, where glial-cell coupling is eliminated, suggesting that K^+ current flow within single glial cells can effectively clear K^+ (Wallraff et al., 2006). This finding is supported by the observation that astrocytes within the stratum radiatum are preferentially oriented in a perpendicular direction (Wallraff et al., 2006).

6.7 Potassium Siphoning and the Regulation of Blood Flow

Neuronal activity evokes localized changes in blood flow, a response termed *functional hyperemia* or *neurovascular coupling*. A consequence of K^+ siphoning is that neuronal activity will lead to an efflux of K^+ from glial cell endfeet onto blood vessels. Paulson and Newman (1987) have proposed that this siphoning mechanism could mediate neurovascular coupling, as modest increases in K^+ at the vessel wall leads to vasodilation. This hypothesis was recently tested in the retina (Metea et al., 2007). Potassium efflux from glial cell endfeet was evoked by depolarizing individual glial cells. Vessels adjacent to the glial cells did not dilate. In addition, light-evoked vasodilations were monitored in transgenic mouse retinas, where Kir4.1, the main glial K^+ channel, was knocked out. Although K^+ siphoning currents are largely absent in glial cells of these animals, light-evoked vasodilations were not reduced. These results demonstrate that, contrary to the hypothesis, K^+ siphoning does not contribute significantly to neurovascular coupling in the retina.

Filosa et al. (2006) have recently proposed that K^+ efflux from glial cell endfeet, mediated by a nonsiphoning mechanism, is responsible for neurovascular coupling. They suggest that neuronal activity results in the opening of Ca^{2+}-activated K^+ (BK) channels in glial endfeet, resulting in the efflux of K^+ onto blood vessels and to vessel dilation.

6.8 Relative Importance of K^+ Regulatory Mechanisms

The relative importance of the different K^+ regulatory mechanisms, including active and passive K^+ uptake, K^+ spatial buffering, and K^+ siphoning, remains uncertain and is a question of considerable debate. It is likely that the relative importance of the mechanisms varies in different CNS regions. In the rat optic nerve, for example, K^+ regulation appears to depend more on active uptake of K^+ than on K^+ spatial buffering as recovery of $[K^+]_o$ following stimulation is highly sensitive to Na^+ pump inhibition but not to glial K^+-channel blockers (Ransom et al., 2000). By contrast, in the CA3 region of rat hippocampus, both the Na^+ pump and glial K^+ channels are critical for maintaining baseline $[K^+]_o$ and for recovery of $[K^+]_o$ following stimulation (D'Ambrosio et al., 2002) (Fig. 6.3). In this preparation, the Na^+ pump is necessary for the clearance of excess K^+ during afferent stimulation while glial K^+ channels are necessary to prevent

large $[K^+]_o$ undershoots following tetanic stimulation (D'Ambrosio et al., 2002). In the CA1 region of the hippocampus, both spatial buffering through glial-cell networks and spatial buffering/siphoning through individual glial cells contribute to $[K^+]_o$ regulation (Wallraff et al., 2006). In the amphibian retina, K^+ spatial buffering and, in particular K^+ siphoning, has a major role in regulating $[K^+]_o$ (Newman, 1995). When glial K^+ channels are blocked pharmacologically, recovery of $[K^+]_o$ following stimulation is prolonged and transfer of K^+ from the retina to the vitreous is blocked.

The factors that determine the relative contribution of each K^+ clearance mechanism remains uncertain. A few general principals have emerged, however. Coupling between astrocytes does not appear to contribute greatly to K^+ clearance as $[K^+]_o$ dynamics are not substantially altered in connexin KO animals. Thus, long-range spatial buffering through the astrocyte syncytium, as originally proposed by Orkand et al. (1966), may not be a dominant K^+ clearance mechanism. In contrast, K^+ siphoning, a specialized form of K^+ spatial buffering, does contribute significantly to K^+ clearance in those CNS regions bordering a large fluid reservoir. Thus, K^+ siphoning is instrumental in clearing K^+ from the retina, which is a thin sheet of CNS tissue surrounded by the vitreous humor and the subretinal space, which both function as sinks where K^+ can be temporarily stored. Passive and active uptake of K^+ may play a more important role in $[K^+]_o$ regulation in those CNS regions where K^+ spatial buffering/siphoning cannot efficiently move K^+ to fluid reservoirs.

6.9 Glial Cells and K^+ Channels

Kir channels most likely underlie K^+ spatial buffering in the CNS and there is considerable interest in determining their macromolecular structure, mechanisms of targeting, and modulation by intracellular and extracellular factors. The Kir channels have been recently cloned, and over 20 genes are currently known to encode various Kir-channel subunits (Nichols and Lopatin, 1997; Stanfield et al., 2002). Site-directed mutagenesis and heterologous channel expression have been used to identify structural elements involved in specific Kir-channel functions. These studies have revealed the basic Kir-channel design of two transmembrane domains and a re-entry loop (P-loop), with intracellular amino and carboxyl termini (Nichols and Lopatin, 1997; Stanfield et al., 2002). The Kir-channel subunits are usually categorized into seven major subfamilies (Kir1 to Kir7) that are diversely regulated by intracellular and extracellular factors (Stanfield et al., 2002).

Of these family members, immunocytochemical and in situ hybridization studies demonstrate that the Kir4.1 channel is broadly expressed in brain (Poopalasundaram et al., 2000; Higashi et al., 2001), though different reports have suggested that it is expressed only in glial cells (Higashi et al., 2001) or in both neurons and glia (Li et al., 2001). Kir4.1 immunoreactivity can be demonstrated in cultured (Li et al., 2001; Kucheryavykh et al., 2007) and in situ astrocytes (Poopalasundaram et al., 2000; Higashi et al., 2001; Olsen et al., 2006) and in oligodendrocytes (Kalsi et al., 2004). In the olfactory bulb, Kir4.1 immunoreactivity is detected in about half of the glial fibrillary acidic protein-positive astrocytes, but not in neurons (Higashi et al., 2001). Immunogold microscopic

examination reveals that Kir4.1 channels are enriched in the processes of astrocytes enveloping synapses and blood vessels (Higashi et al., 2001). In addition to Kir4.1 channels, other Kir-channel subunits may also be expressed in various glial-cell types. Kir2.2 channels are expressed in Bergmann glial cells and astrocytes in the cerebellum (Leonoudakis et al., 2001) and Kir2.1 channels are found in astrocytes and oligodendrocytes in the forebrain (Stonehouse et al., 1999). Single-cell in situ PCR experiments in the astrocytes from mouse hippocampal slices have identified transcripts for Kir2.1, Kir2.2, Kir2.3 and Kir4.1 channels (Schroder et al., 2002). Thus, a large variety of Kir-channel subtypes may be expressed in glial cells and this may explain the wide range of single-channel Kir conductances reported in glial cells (Sontheimer, 1994).

TASK and TREK channels are members of the two-pore domain potassium-channel family and form either homomeric or heteromeric open-rectifier (leak) channels (Patel and Honore, 2001). Recent evidence suggests that these channels are expressed in macroglial cells, including astrocytes (Rusznak et al., 2004; Gnatenco et al., 2002; Kindler et al., 2000) and Müller cells (Skatchkov et al., 2006). The degree to which these channels contribute to $[K^+]_o$ regulation remains to be determined.

The properties of specific Kir-channel subunits have been assessed in heterologous expression systems such as *Xenopus* oocytes and transfected cells. Kir2.1 currents show steep inwardly rectifying current–voltage relationships with minimal outward currents at membrane potentials positive to E_K (Kubo et al., 1993). In contrast, Kir4.1 channels are weakly rectifying, allowing substantial outward currents (Takumi et al., 1995). A further complexity is provided by the fact that Kir channels are tetrameric proteins, and in heterologous expression systems Kir subunits can form either homomeric or heteromeric channels (Stanfield et al., 2002). The expression of Kir5.1 subunits in *Xenopus* oocytes or mammalian cell lines does not result in functional channels, but coexpression with Kir4.1 channels leads to formation of heteromeric channels that are highly sensitive to intracellular changes in pH (Tucker et al., 2000).

As in retinal Müller cells (see below), the distribution of K^+ conductance in astrocytes is nonhomogenous. Freshly dissociated salamander astrocytes have an approximately tenfold higher conductance in their endfeet than in other cell regions (Newman, 1986). In mammals, only Kir4.1 channels have been mapped to astrocytic endfeet (Higashi et al., 2001). Although it is clear that K^+ conductance in astrocytes is likely mediated by Kir channels, further investigations are required to determine the precise molecular composition of such channels.

6.10 Kir-Channel Subtypes Expressed in Müller Cells

In contrast to astrocytes in the brain, retinal Müller cells are a relatively homogenous class of cells and are thus an attractive model for studying Kir-channel composition. Several groups have examined the distribution of Kir channels in Müller cells using immunocytochemical and molecular biological techniques (Ishii et al., 1997; Kofuji

et al., 2000). These studies demonstrate highly concentrated expression of the weakly rectifying Kir4.1 channels in Müller cell endfeet and on the processes enveloping blood vessels (Fig. 6.6a,c,e), a distribution that correlates well with the previously mentioned electrophysiological studies (Newman, 1987b, 1993). Several additional

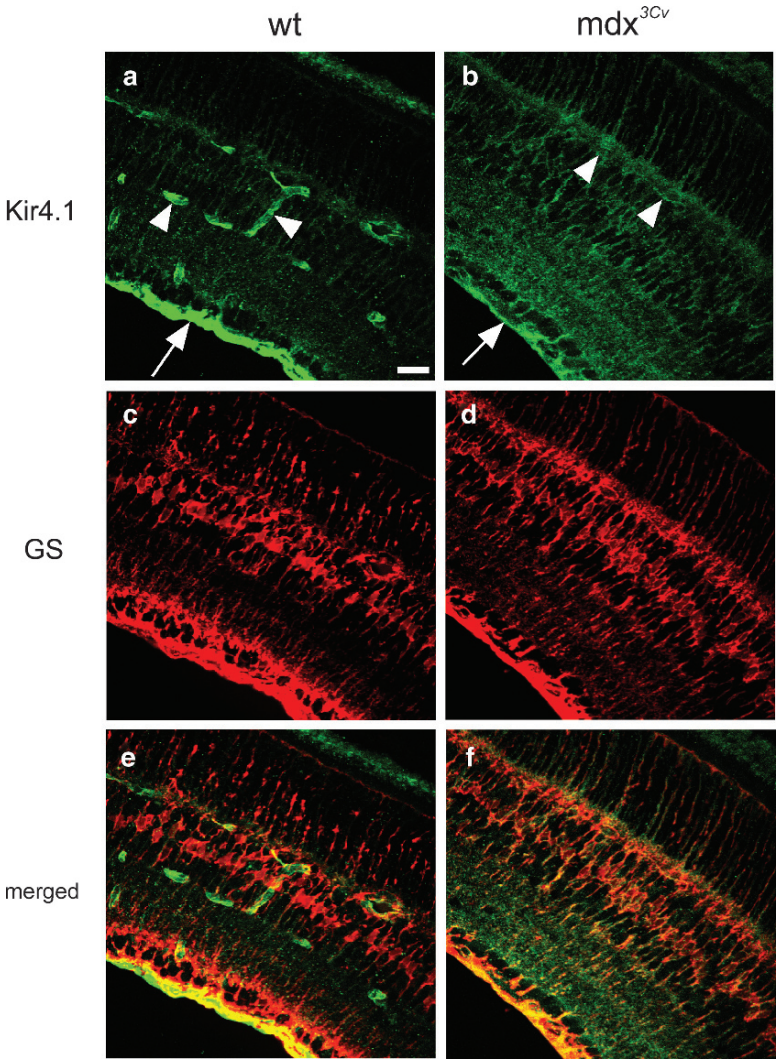

Fig. 6.6 Kir4.1-channel localization in wild type and mdx3Cv (dystrophin knockout) mouse retinas. (**a**) In the wild-type retina, Kir4.1 is concentrated at the inner limiting membrane (*arrow*) and to processes surrounding blood vessels (*arrowheads*). (**b**) In the mdx3Cv retina, Kir4.1 is more evenly distributed throughout the retina with a reduction in staining at the inner limiting membrane (*arrow*) and no apparent enrichment of Kir4.1 around blood vessels (*arrowheads*). The glial-cell marker glutamine synthetase (GS) (**c**, **d**), and merged images (**e**, **f**) suggest that Kir4.1 is localized to Müller cells. Scale bar in (**a**) = 25 μm. [From Connors and Kofuji (2002), with permission.] (*See Color Plates*).

lines of evidence argue that Kir4.1 channels are the principal Kir-channel subtype in Müller cells: (1) patch-clamp recordings in rabbit Müller cells and in transfected 293 cells expressing Kir4.1 channels show similar single-channel conductances and open probabilities (Tada et al., 1998); (2) genetic ablation of Kir4.1 channels in mice decreases the membrane conductance of Müller cells by 13–77-fold (Kofuji et al., 2000; Metea et al., 2007); and (3) the slow PIII wave of the electroretinogram, which is generated by K^+ fluxes through Müller cells, is absent in Kir4.1 KO animals (Kofuji et al., 2000). This effect is not caused by overall impairment of neuronal function as indicated by the fact that the a and b waves, associated with neuronal activity, are not decreased in the KO animals.

Other Kir channels, such as the strongly rectifying Kir2.1 channels, may also be expressed in Müller cells. In mouse Müller cells, Kir2.1 channels are expressed along the plasma membrane in a uniform manner that does not resemble the clustered distribution seen for Kir4.1 channels (Kofuji et al., 2002). Such differential expression of Kir2.1 and Kir4.1 channels may enhance the efficiency of K^+ siphoning in the retina (Kofuji et al., 2002). Expression of weakly rectifying Kir4.1 channels in selective membrane domains would allow K^+ ions to leave Müller cells and be stored in extracellular sinks such as the vitreous humor, whereas expression of strongly rectifying Kir2.1 channels would allow greater influx of K^+ in the synaptic layers (Kofuji et al., 2002).

An additional study suggests the expression of Kir5.1 channels in the soma and stalks of Müller cells; immunoprecipitation assays show that a fraction of the Kir4.1 subunits in retina are coassembled with Kir5.1 subunits (Ishii et al., 2003). Because heterologously expressed Kir4.1 and Kir5.1 form heteromeric Kir channels that are highly sensitive to physiological changes in intracellular pH, it has been suggested that the expression of Kir5.1 subunits in Müller cells promotes coordinated coupling between acid–base regulation and K^+ buffering in the retina (Ishii et al., 2003). In this hypothesis, increases in extracellular K^+ concentration and the resulting glial depolarization would increase the activity of the electrogenic Na^+-HCO_3^- cotransporter (Newman, 1996a). The increased influx of HCO_3^- would then cause intracellular alkalinization and subsequent increases in the activity of heteromeric Kir4.1/Kir5.1 channels, ultimately enhancing K^+ uptake into Müller cells (Ishii et al., 2003).

In summary, investigations in Müller cells have provided compelling evidence, demonstrating a major role for Kir4.1 channels in retinal K^+ regulation. Kir4.1 channels appear to have the functional and anatomical distributions that best match the physiological studies performed in Müller cells over the past decade. Biochemical and immunocytochemical work also suggests that other Kir channels, including Kir2.1 and Kir5.1, may be involved in K^+ influx in these cells.

6.11 Kir-Channel Accessory Proteins in Müller Cells: Localization and Function

The focal aggregation of Kir4.1 channels in Müller cells raises the intriguing question of how these channels are targeted to such precise subcellular domains. This is an important question as the efficiency of the retinal K^+ siphoning process is highly

dependent on the clustered, nonhomogenous distribution of Kir channels in Müller cells (Newman, 1995). The water-channel aquaporin 4 (AQP4) is also highly enriched in the endfeet and perivascular processes of Müller cells (Nagelhus et al., 1999). This spatial overlap of Kir and aquaporins, two highly nonhomologous channel types, suggests that there may be a common molecular mechanism for their subcellular distribution and targeting. Although the Kir4.1 and AQP4 channels are highly divergent in their primary sequences, they share a key –S–X–V–COOH motif in their C termini. This sequence is able to bind to PDZ domains, which are modular amino acid motifs implicated in many protein–protein interactions (Hung and Sheng, 2002). Proteins possessing these domains are abundantly expressed in the nervous system and include postsynaptic density protein-95 (PSD-95), Chapsyn-110/PSD-93, SAP-102, and hDlg/SAP97 (Hung and Sheng, 2002). Positioning of several proteins in the postsynaptic density in excitatory synapses are critically dependent on their interactions with PDZ-domain containing proteins (Sheng and Sala, 2001).

Although specific PDZ domain-containing protein(s) are yet to be unequivocally identified in Müller cells, the SAP97 protein, which is present in Müller cells, has been shown to increase Kir4.1 currents in heterologous systems (Horio et al., 1997). Another candidate is the PDZ domain-containing adapter protein, α-syntrophin. In tissues where α-syntrophin is present, it is localized to the cell membrane by its association with the multiprotein dystrophin glycoprotein complex (DGC) (Ahn and Kunkel, 1995). The DGC spans the cell membrane, forming a molecular bridge between basal lamina proteins in the extracellular space and an array of signaling molecules in the intracellular domain. Immunolocalization studies in retina revealed that the DGC components, α-dystroglycan and the short dystrophin isoform, Dp71, appear to be localized in a fashion very similar to that of Kir4.1 in Müller cells (Claudepierre et al., 2000b). The role of Dp71 in the localization of Kir4.1 has been investigated in the dystrophin null mutant mouse, mdx^{3Cv} (Connors and Kofuji, 2002). Immunohistochemistry experiments reveal that the polarized subcellular distribution of Kir4.1 is altered in Müller glial cells from mdx^{3Cv} mice, displaying a more homogeneous distribution pattern (Fig. 6.6b,d,f). Immunoblotting and whole cell patch clamp experiments reveal that the channel is expressed at normal levels at the plasma membrane and its electrophysiological properties are unchanged (Connors and Kofuji, 2002). Similar findings have also been reported for a null mouse line for the dystrophin isoform Dp71 (Dalloz et al, 2003).

These results strongly suggest that the DGC is important to the localization of Kir4.1 in Müller cells. It is possible that the DGC targets the Kir channels to the membrane domains facing the vitreous and blood vessels by binding of extracellular portions of the DGC to the basal lamina of these regions (Fig. 6.7). This assumes the existence of an intermediate protein containing a PDZ domain. As mentioned previously, the best candidate for such an adaptor protein is α-syntrophin. α-syntrophin is expressed in Müller cells and is putatively part of the Müller cell-specific DGC (Claudepierre et al., 2000a). In addition, a-syntrophin has been shown to interact with AQP4 in astrocytes in a PDZ-dependent manner, and is required for membrane expression and localization of AQP4 (Neely et al., 2001). Therefore, α-syntrophin could underlie the colocalization of Kir4.1 and AQP4 seen

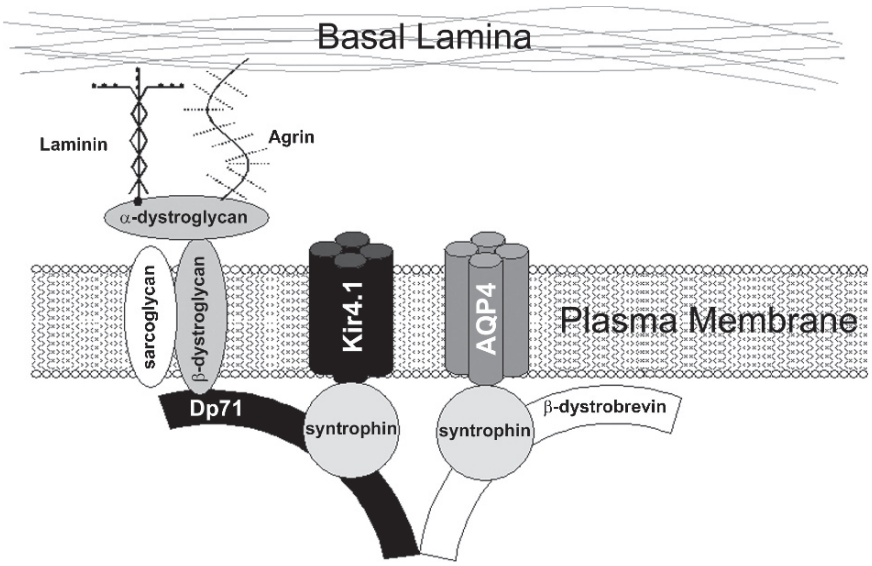

Fig. 6.7 Schematic representation of the glial-cell dystrophin–glycoprotein complex (DGC) and associated Kir and aquaporin channels. The dystrophin–glycoprotein complex is shown with its putative interactions with a syntrophin isoform, Kir4.1 and AQP4. [From Kofuji and Connors (2003), with permission.]

in Müller cells. The importance of laminin in the clustering of Kir4.1 channels has been demonstrated in a study showing that laminin, agrin, and α-dystroglycan codistribute with Kir4.1 in Müller cell endfeet (Noel et al., 2005). In cultured Müller cells, addition of laminin-1 induces the clustering of α-dystroglycan and Kir4.1.

In astrocytes, the direct participation of DGC proteins in the targeting of Kir4.1 and AQP4 channels has been demonstrated in an α-syntrophin KO mouse line. In astrocytes, as in Müller cells, AQP4 and Kir4.1 are strongly expressed in glial endfeet that are in direct contact with capillaries and the pia (Nielsen et al., 1997; Higashi et al., 2001). Quantitative immunoelectromicroscopy has shown that in the hippocampus of α-syntrophin KO mice, the expression of AQP4 in astrocyte endfeet is greatly diminished, while expression of Kir4.1 channels is less affected (Amiry-Moghaddam et al., 2003). These results indicate that α-syntrophin is critical for the targeting and clustering of AQP4 channels to astrocytic endfeet, but is perhaps not as vital for targeting of Kir4.1 channels. Further work is needed to establish whether Kir4.1 channels are indeed linked to the DGC in astrocytes via syntrophin isoforms.

Despite the apparent lack of major rearrangements in Kir4.1-channel localization and expression in hippocampus astrocytes, the clearance of extracellular K^+ following neuronal stimulation is slowed twofold in hippocampal slices from α-syntrophin KO mice (Amiry-Moghaddam et al., 2003). This study suggests that AQP4 plays an essential role in K^+ clearance by the K^+ spatial buffering mechanism. In support of

this view, the clearance of K^+ following spreading depression is slowed twofold in AQP4 null mice (Padmawar et al., 2005). As suggested by Ottersen and colleagues (Nagelhus et al., 1999; Amiry-Moghaddam et al., 2003), the transfer of K^+ ions across the plasma membrane of glial cells by K^+ spatial buffering generates osmotic imbalances. Water fluxes paralleling the K^+ flow are needed to dissipate the osmotic imbalance. In the absence of AQP4 channels in regions where Kir4.1-channel density is high, spatial buffering cannot precede efficiently.

These tantalizing observations suggest that impaired targeting or function of AQP4 and Kir4.1 channels may have clinical relevance in conditions such as epilepsy or brain edema. It remains to be seen, however, how significant a role Kir4.1 channels play in K^+ spatial buffering in the brain and in the overall regulation of $[K^+]_o$. Future studies in mice with genetic inactivation of Kir4.1 channels should provide an answer to this important question.

6.12 Impaired Potassium Regulation in Pathological Conditions

In many types of pathology, $[K^+]_o$ regulation is likely to be impaired by downregulation of Kir channels in glial cells. For example, in patients with temporal lobe epilepsy there is marked reduction of Kir currents in astrocytes (Bordey and Sontheimer, 1998; Hinterkeuser et al., 2000), which could contribute to the increased excitability of the epileptic tissue. Indeed, there is a reduction of $[K^+]_o$ clearance in sclerotic tissue from epileptic hippocampus in comparison to nonsclerotic tissue (Heinemann et al., 2000). In animal models of retinal ischemia and diabetes, there is also downregulation and/or mislocalization of expression of Kir4.1 channels in Müller cells (Pannicke et al., 2006); (Iandiev et al., 2006). Similar loss of Kir4.1 expression is seen in an animal model of amyotrophic lateral sclerosis (Kaiser et al., 2006). Overall, such changes of Kir expression in macroglial cells are expected to impair the rapid movement of K^+ and water in these cells and may contribute to the pathophysiology associated with these disorders.

6.13 Conclusions

It has been over 40 years since Orkand et al. (1966) initially proposed the K^+ spatial buffering mechanism of $[K^+]_o$ regulation in the CNS. Since then, it has become clear that $[K^+]_o$ regulation involves both net uptake of K^+ and K^+ spatial buffering. The relative importance of these K^+ homeostatic mechanisms may vary from site to site in the CNS. Potassium spatial buffering has been carefully characterized in the retina, where a specialized form of spatial buffering, K^+ siphoning, directs K^+ from the plexiform layers to the vitreous humor and to blood vessels (Newman et al., 1984). Molecular studies indicate that Kir4.1-channel localization is critical to the highly asymmetric K^+ conductance found in Müller cells (Kofuji et al., 2000).

Furthermore, Kir2.1 and possibly Kir5.1 are also expressed in Müller cells (Kofuji et al., 2002). Potassium siphoning in the retina may be facilitated by concerted action of the strongly rectifying Kir2.1 channels, which allow K^+ entry into Müller cells, and the weakly rectifying Kir4.1 channels, which allow K^+ exit to large sinks such as the vitreous humor (Kofuji et al., 2002). Moreover, dystrophin and dystrophin-associated proteins may promote the clustering and subcellular distribution of Kir4.1 channels and the water channel AQP4 in astrocytes (Amiry-Moghaddam et al., 2003). The colocalization of water and K^+ channels in glial-cell membranes suggests that K^+ buffering and water flux are tightly coupled in the brain.

Although significant progress has been made in the past decade, we still do not have a coherent picture of the relative importance of the various mechanisms contributing to $[K^+]_o$ regulation in the CNS. Given recent advances in optical, electrophysiological, and genetic methods, it is plausible that this picture will gain greater clarity in the near future.

References

Ahn AH, Kunkel LM (1995) Syntrophin binds to an alternatively spliced exon of dystrophin. *J Cell Biol* 128:363–371.

Amedee T, Robert A, Coles JA (1997) Potassium homeostasis and glial energy metabolism. *Glia* 21:46–55.

Amiry-Moghaddam M, Otsuka T, Hurn PD, Traystman RJ, Haug FM, Froehner SC, Adams ME, Neely JD, Agre P, Ottersen OP, Bhardwaj A (2003) An -syntrophin-dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. *Proc Natl Acad Sci U S A* 100:2106–2111.

Ballanyi K, Grafe P, ten Bruggencate G (1987) Ion activities and potassium uptake mechanisms of glial cells in guinea-pig olfactory cortex slices. *J Physiol* 382:159–174.

Barres BA, Koroshetz WJ, Chun LL, Corey DP (1990) Ion channel expression by white matter glia: the type-1 astrocyte. *Neuron* 5:527–544.

Bordey A, Sontheimer H (1998) Properties of human glial cells associated with epileptic seizure foci. *Epilepsy Res* 32:286–303.

Brew H, Gray P, Mobbs P, Attwell D (1986) Endfeet of retinal glial cells have higher densities of ion channels that mediate K+ buffering. *Nature* 324:466–468.

Claudepierre T, Dalloz C, Mornet D, Matsumura K, Sahel J, Rendon A (2000a) Characterization of the intermolecular associations of the dystrophin-associated glycoprotein complex in retinal Müller glial cells. *J Cell Sci 113 (Pt* 19):3409–3417.

Claudepierre T, Mornet D, Pannicke T, Forster V, Dalloz C, Bolanos F, Sahel J, Reichenbach A, Rendon A (2000b) Expression of Dp71 in Müller glial cells: a comparison with utrophin- and dystrophin-associated proteins. *Invest Ophthalmol Vis Sci* 41:294–304.

Coles JA, Orkand RK, Yamate CL, Tsacopoulos M (1986) Free concentrations of Na, K, and Cl in the retina of the honeybee drone: stimulus-induced redistribution and homeostasis. *Ann N Y Acad Sci* 481:303–317.

Connors B, Dray A, Fox P, Hilmy M, Somjen G (1979) LSD's effect on neuron populations in visual cortex gauged by transient responses of extracellular potassium evoked by optical stimuli. *Neurosci Lett* 13:147–150.

Connors BW, Ransom BR, Kunis DM, Gutnick MJ (1982) Activity-dependent K+ accumulation in the developing rat optic nerve. *Science* 216:1341–1343.

Connors NC, Kofuji P (2002) Dystrophin Dp71 is critical for the clustered localization of potassium channels in retinal glial cells. *J Neurosci* 22:4321–4327.

D'Ambrosio R, Gordon DS, Winn HR (2002) Differential role of KIR channel and Na+/K+-pump in the regulation of extracellular K^+ in rat hippocampus. *J Neurophysiol* 87:87–102.

Dalloz C, Sarig R, Fort P, Yaffe D, Bordais A, Pannicke T, Grosche J, Mornet D, Reichenbach A, Sahel J, Nudel U, Rendon A (2003) Targeted inactivation of dystrophin gene product Dp71: phenotypic impact in mouse retina. *Hum Mol Genet* 12:1543–1554.

Dennis MJ, Gerschenfeld HM (1969) Some physiological properties of identified mammalian neuroglial cells. *J Physiol* 203:211–222.

Dermietzel R (1998) Diversification of gap junction proteins (connexins) in the central nervous system and the concept of functional compartments. *Cell Biol Int* 22:719–730.

Dermietzel R, Gao Y, Scemes E, Vieira D, Urban M, Kremer M, Bennett MV, Spray DC (2000) Connexin43 null mice reveal that astrocytes express multiple connexins. *Brain Res Brain Res Rev* 32:45–56.

Dietzel I, Heinemann U, Hofmeier G, Lux HD (1980) Transient changes in the size of the extracellular space in the sensorimotor cortex of cats in relation to stimulus-induced changes in potassium concentration. *Exp Brain Res* 40:432–439.

Dietzel I, Heinemann U, Lux HD (1989) Relations between slow extracellular potential changes, glial potassium buffering, and electrolyte and cellular volume changes during neuronal hyperactivity in cat brain. *Glia* 2:25–44.

Doupnik CA, Davidson N, Lester HA (1995) The inward rectifier potassium channel family. *Curr Opin Neurobiol* 5:268–277.

Filosa JA, Bonev AD, Straub SV, Meredith AL, Wilkerson MK, Aldrich RW, Nelson MT (2006) Local potassium signaling couples neuronal activity to vasodilation in the brain. *Nat Neurosci* 9:1397–1403.

Franck G, Grisar T, Moonen G (1983) Glial and neuronal Na^+,K^+ pump. In: Advances in Neurobiology (Fedoroff S, Hertz L, eds), pp 139–159. New York: Academic.

Frishman LJ, Yamamoto F, Bogucka J, Steinberg RH (1992) Light-evoked changes in $[K^+]o$ in proximal portion of light-adapted cat retina. *J Neurophysiol* 67:1201–1212.

Gardner-Medwin AR, Nicholson C (1983) Changes of extracellular potassium activity induced by electric current through brain tissue in the rat. *J Physiol* 335:375–392.

Gardner-Medwin AR, Coles JA, Tsacopoulos M (1981) Clearance of extracellular potassium: evidence for spatial buffering by glial cells in the retina of the drone. *Brain Res* 209:452–457.

Gnatenco C, Han J, Snyder AK, Kim D (2002) Functional expression of TREK-2 K^+ channel in cultured rat brain astrocytes. *Brain Res* 931:56–67.

Gutnick MJ, Heinemann U, Lux HD (1979) Stimulus induced and seizure related changes in extracellular potassium concentration in cat thalamus (VPL). *Electroencephalogr Clin Neurophysiol* 47:329–344.

Haas M, Forbush B, III (1998) The Na-K-Cl cotransporters. *J Bioenerg Biomembr* 30:161–172.

Heinemann U, Lux HD (1977) Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. *Brain Res* 120:231–249.

Heinemann U, Schaible HG, Schmidt RF (1990) Changes in extracellular potassium concentration in cat spinal cord in response to innocuous and noxious stimulation of legs with healthy and inflamed knee joints. *Exp Brain Res* 79:283–292.

Heinemann U, Gabriel S, Jauch R, Schulze K, Kivi A, Eilers A, Kovacs R, Lehmann TN (2000) Alterations of glial cell function in temporal lobe epilepsy. *Epilepsia* 41:S185–S189.

Higashi K, Fujita A, Inanobe A, Tanemoto M, Doi K, Kubo T, Kurachi Y (2001) An inwardly rectifying K+ channel, Kir4.1, expressed in astrocytes surrounds synapses and blood vessels in brain. *Am J Physiol Cell Physiol* 281:C922–C931.

Hinterkeuser S, Schroder W, Hager G, Seifert G, Blumcke I, Elger CE, Schramm J, Steinhauser C (2000) Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. *Eur J Neurosci* 12:2087–2096.

Holthoff K, Witte OW (2000) Directed spatial potassium redistribution in rat neocortex. *Glia* 29:288–292.

Horio Y, Hibino H, Inanobe A, Yamada M, Ishii M, Tada Y, Satoh E, Hata Y, Takai Y, Kurachi Y (1997) Clustering and enhanced activity of an inwardly rectifying potassium channel, Kir4.1, by an anchoring protein, PSD-95/SAP90. *J Bio Chem* 272:12885–12888.

Hung AY, Sheng M (2002) PDZ domains: structural modules for protein complex assembly. *J Biol Chem* 277:5699–5702.

Iandiev I, Tenckhoff S, Pannicke T, Biedermann B, Hollborn M, Wiedemann P, Reichenbach A, Bringmann A (2006) Differential regulation of Kir4.1 and Kir2.1 expression in the ischemic rat retina. *Neurosci Lett* 396:97–101.

Ishii M, Horio Y, Tada Y, Hibino H, Inanobe A, Ito M, Yamada M, Gotow T, Uchiyama Y, Kurachi Y (1997) Expression and clustered distribution of an inwardly rectifying potassium channel, KAB-2/Kir4.1, on mammalian retinal Müller cell membrane: their regulation by insulin and laminin signals. *J Neurosci* 17:7725–7735.

Ishii M, Fujita A, Iwai K, Kusaka S, Higashi K, Inanobe A, Hibino H, Kurachi Y (2003) Differential expression and distribution of Kir5.1 and Kir4.1 inwardly rectifying K^+ channels in retina. *Am J Physiol Cell Physiol* 285:C260–267.

Jorgensen PL, Hakansson KO, Karlish SJ (2003) Structure and mechanism of Na,K-ATPase: functional sites and their interactions. *Annu Rev Physiol* 65:817–849.

Kaiser M, Maletzki I, Hulsmann S, Holtmann B, Schulz-Schaeffer W, Kirchhoff F, Bahr M, Neusch C (2006) Progressive loss of a glial potassium channel (KCNJ10) in the spinal cord of the SOD1 (G93A) transgenic mouse model of amyotrophic lateral sclerosis. *J Neurochem* 99:900–912.

Kalsi AS, Greenwood K, Wilkin G, Butt AM (2004) Kir4.1 expression by astrocytes and oligodendrocytes in CNS white matter: a developmental study in the rat optic nerve. *J Anat* 204:475–485.

Kaplan JH (2002) Biochemistry of Na,K-ATPase. *Annu Rev Biochem* 71:511–535.

Karwoski CJ, Xu X (1999) Current source-density analysis of light-evoked field potentials in rabbit retina. *Vis Neurosci* 16:369–377.

Karwoski CJ, Newman EA, Shimazaki H, Proenza LM (1985) Light-evoked increases in extracellular K+ in the plexiform layers of amphibian retinas. *J Gen Physiol* 86:189–213.

Karwoski CJ, Lu HK, Newman EA (1989) Spatial buffering of light-evoked potassium increases by retinal Müller (glial) cells. *Science* 244:578–580.

Katzman R (1976) Maintenance of a constant brain extracellular potassium. *Fed Proc* 35:1244–1247.

Kimelberg HK, Frangakis MV (1985) Furosemide- and bumetanide-sensitive ion transport and volume control in primary astrocyte cultures from rat brain. *Brain Res* 361:125–134.

Kindler CH, Pietruck C, Yost CS, Sampson ER, Gray AT (2000) Localization of the tandem pore domain K+ channel TASK-1 in the rat central nervous system. *Brain Res Mol Brain Res* 80:99–108.

Kofuji P, Connors NC (2003) Molecular substrates of potassium spatial buffering in glial cells. *Mol Neurobiol* 28:195–208.

Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. *Neuroscience* 129:1045–1056.

Kofuji P, Ceelen P, Zahs KR, Surbeck LW, Lester HA, Newman EA (2000) Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. *J Neurosci* 20:5733–5740.

Kofuji P, Biedermann B, Siddharthan V, Raap M, Iandiev I, Milenkovic I, Thomzig A, Veh RW, Bringmann A, Reichenbach A (2002) Kir potassium channel subunit expression in retinal glial cells: implications for spatial potassium buffering. *Glia* 39:292–303.

Kubo Y, Baldwin TJ, Jan YN, Jan LY (1993) Primary structure and functional expression of a mouse inward rectifier potassium channel. *Nature* 362:127–133.

Kucheryavykh YV, Kucheryavykh LY, Nichols CG, Maldonado HM, Baksi K, Reichenbach A, Skatchkov SN, Eaton MJ (2007) Downregulation of Kir4.1 inward rectifying potassium channel subunits by RNAi impairs potassium transfer and glutamate uptake by cultured cortical astrocytes. *Glia* 55:274–281.

Kuffler SW, Nicholls JG, Orkand RK (1966) Physiological properties of glial cells in the central nervous system of amphibia. *J Neurophysiol* 29:768–787.

Kume-Kick J, Mazel T, Vorisek I, Hrabetova S, Tao L, Nicholson C (2002) Independence of extracellular tortuosity and volume fraction during osmotic challenge in rat neocortex. *J Physiol* 542:515–527.

Leonoudakis D, Mailliard W, Wingerd K, Clegg D, Vandenberg C (2001) Inward rectifier potassium channel Kir2.2 is associated with synapse-associated protein SAP97. *J Cell Sci* 114:987–998.

Li L, Head V, Timpe LC (2001) Identification of an inward rectifier potassium channel gene expressed in mouse cortical astrocytes. *Glia* 33:57–71.

MacVicar BA, Feighan D, Brown A, Ransom B (2002) Intrinsic optical signals in the rat optic nerve: role for K(+) uptake via NKCC1 and swelling of astrocytes. *Glia* 37:114–123.

Matthias K, Kirchhoff F, Seifert G, Huttmann K, Matyash M, Kettenmann H, Steinhauser C (2003) Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. *J Neurosci* 23:1750–1758.

Metea MR, Kofuji P, Newman EA (2007) Neurovascular coupling is not mediated by potassium siphoning from glial cells. *J Neurosci* 27:2468–2471.

Nagelhus E, Horio Y, Inanobe A, Fujita A, Haug F, Nielsen S, Kurachi Y, Ottersen O (1999) Immnunogold evidence suggests that coupling of K^+ siphoning and water transport in rat retinal Müller cells is mediated by coenrichment of Kir4.1 and AQP4 in specific membrane domains. *Glia* 26:47–54.

Nagy JI, Rash JE (2000) Connexins and gap junctions of astrocytes and oligodendrocytes in the CNS. *Brain Res Brain Res Rev* 32:29–44.

Neely JD, Amiry-Moghaddam M, Ottersen OP, Froehner SC, Agre P, Adams ME (2001) Syntrophin-dependent expression and localization of Aquaporin-4 water channel protein. *Proc Natl Acad Sci U S A* 98:14108–14113.

Neusch C, Papadopoulos N, Muller M, Maletzki I, Winter SM, Hirrlinger J, Handschuh M, Bahr M, Richter DW, Kirchhoff F, Hulsmann S (2006) Lack of the Kir4.1 channel subunit abolishes K+ buffering properties of astrocytes in the ventral respiratory group: impact on extracellular K+ regulation. *J Neurophysiol* 95:1843–1852.

Newman EA (1984) Regional specialization of retinal glial cell membrane. *Nature* 309:155–157.

Newman EA (1985) Membrane physiology of retinal glial (Müller) cells. *J Neurosci* 5:2225–2239.

Newman EA (1986) High potassium conductance in astrocyte endfeet. *Science* 233:453–454.

Newman EA (1987a) Regulation of potassium levels by Müller cells in the vertebrate retina. *Can J Phys & Pharm* 65:1028–1034.

Newman EA (1987b) Distribution of potassium conductance in mammalian Müller (glial) cells: a comparative study. *J Neurosci* 7:2423–2432.

Newman EA (1993) Inward-rectifying potassium channels in retinal glial (Müller) cells. *J Neurosci* 13:3333–3345.

Newman EA (1995) Glial cell regulation of extracellular potassium. In: Neuroglia (Kettenmann H, Ransom B, eds), pp 717–731. New York: Oxford University Press.

Newman EA (1996a) Acid efflux from retinal glial cells generated by sodium bicarbonate cotransport. *J Neurosci* 16:159–168.

Newman EA (1996b) Regulation of extracellular K^+ and pH by polarized ion fluxes in glial cells: the retinal Muller cell. *The Neuroscientist* 2:109–117.

Newman EA, Reichenbach A (1996) The Müller cell: a functional element of the retina. *Trends Neurosci* 19:307–312.

Newman EA, Frambach DA, Odette LL (1984) Control of extracellular potassium levels by retinal glial cell K^+ siphoning. *Science* 225:1174–1175.

Nichols CG, Lopatin AN (1997) Inward rectifier potassium channels. *Annu Rev Physiol* 59:171–191.

Nicholson C, Sykova E (1998) Extracellular space structure revealed by diffusion analysis. *Trends Neurosci* 21:207–215.

Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP (1997) Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci* 17:171–180.

Noel G, Belda M, Guadagno E, Micoud J, Klocker N, Moukhles H (2005) Dystroglycan and Kir4.1 coclustering in retinal Müller glia is regulated by laminin-1 and requires the PDZ-ligand domain of Kir4.1. *J Neurochem* 94:691–702.

Oakley BI, Katz B, Xu Z, Zheng J (1992) Spatial buffering of extracellular potassium by Müller (glial) cells in the toad retina. *Exp Eye Res* 55:539–550.

Olsen ML, Higashimori H, Campbell SL, Hablitz JJ, Sontheimer H (2006) Functional expression of Kir4.1 channels in spinal cord astrocytes. *Glia* 53:516–528.

Orkand RK (1986) Glial–interstitial fluid exchange. *Ann N Y Acad Sci* 481:269–272.

Orkand RK, Nicholls JG, Kuffler SW (1966) Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J Neurophysiol* 29:788–806.

Padmawar P, Yao X, Bloch O, Manley GT, Verkman AS (2005) K^+ waves in brain cortex visualized using a long-wavelength K^+-sensing fluorescent indicator. *Nature Methods* 2:825–827.

Pannicke T, Iandiev I, Wurm A, Uckermann O, vom Hagen F, Reichenbach A, Wiedemann P, Hammes HP, Bringmann A (2006) Diabetes alters osmotic swelling characteristics and membrane conductance of glial cells in rat retina. *Diabetes* 55:633–639.

Patel AJ, Honore E (2001) Properties and modulation of mammalian 2P domain K^+ channels. *Trends Neurosci* 24:339–346.

Paulson OB, Newman EA (1987) Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? *Science* 237:896–898.

Poopalasundaram S, Knott C, Shamotienko OG, Foran PG, Dolly JO, Ghiani CA, Gallo V, Wilkin GP (2000) Glial heterogeneity in expression of the inwardly rectifying K^+ channel, Kir4.1, in adult rat CNS. *Glia* 30:362–372.

Ransom BR, Sontheimer H (1992) The neurophysiology of glial cells. *J Clin Neurophysiol* 9:224–251.

Ransom BR, Carlini WG, Connors BW (1986) Brain extracellular space: developmental studies in rat optic nerve. *Ann N Y Acad Sci* 481:87–105.

Ransom C, Sontheimer H, Janigro D (1996) Astrocytic inwardly rectifying potassium currents are dependent on external sodium ions. *J Neurophys* 76:626–630.

Ransom CB, Sontheimer H (1995) Biophysical and pharmacological characterization of inwardly rectifying K+ currents in rat spinal cord astrocytes. *J Neurophysiol* 73:333–346.

Ransom CB, Ransom BR, Sontheimer H (2000) Activity-dependent extracellular K^+ accumulation in rat optic nerve: the role of glial and axonal Na^+ pumps. *J Physiol* 522:427–442.

Reichenbach A, Henke A, Eberhardt W, Reichelt W, Dettmer D (1992) K^+ ion regulation in retina. *Can J Physiol Pharmacol* 70 (Suppl):S239–247.

Rose CR, Ransom BR (1996) Mechanisms of H^+ and Na^+ changes induced by glutamate, kainate, and d-aspartate in rat hippocampal astrocytes. *J Neurosci* 16:5393–5404.

Rouach N, Avignone E, Meme W, Koulakoff A, Venance L, Blomstrand F, Giaume C (2002) Gap junctions and connexin expression in the normal and pathological central nervous system. *Biol Cell* 94:457–475.

Rusznak Z, Pocsai K, Kovacs I, Por A, Pal B, Biro T, Szucs G (2004) Differential distribution of TASK-1, TASK-2 and TASK-3 immunoreactivities in the rat and human cerebellum. *Cell Mol Life Sci* 61:1532–1542.

Schroder W, Seifert G, Huttmann K, Hinterkeuser S, Steinhauser C (2002) AMPA receptor-mediated modulation of inward rectifier K^+ channels in astrocytes of mouse hippocampus. *Mol Cell Neurosci* 19:447–458.

Sheng M, Sala C (2001) PDZ domains and the organization of supramolecular complexes. *Annu Rev Neurosci* 24:1–29.

Singer W, Lux HD (1975) Extracellular potassium gradients and visual receptive fields in the cat striate cortex. *Brain Res* 96:378–383.

Skatchkov SN, Eaton MJ, Shuba YM, Kucheryavykh YV, Derst C, Veh RW, Wurm A, Iandiev I, Pannicke T, Bringmann A, Reichenbach A (2006) Tandem-pore domain potassium channels are functionally expressed in retinal (Muller) glial cells. *Glia* 53:266–276.

Somjen GG (1979) Extracellular potassium in the mammalian central nervous system. *Annu Rev Physiol* 41:159–177.
Somjen GG (2001) Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. *Physiol Rev* 81:1065–1096.
Somjen GG (2002) Ion regulation in the brain: implications for pathophysiology. *Neuroscientist* 8:254–267.
Sontheimer (1994) Voltage-dependent ion channels in glial cells. *Glia* 11:156–172.
Stanfield PR, Nakajima S, Nakajima Y (2002) Constitutively active and G-protein coupled inward rectifier K+ channels: Kir2.0 and Kir3.0. *Rev Physiol Biochem Pharmacol* 145:47–179.
Stonehouse AH, Pringle JH, Norman RI, Stanfield PR, Conley EC, Brammar WJ (1999) Characterisation of Kir2.0 proteins in the rat cerebellum and hippocampus by polyclonal antibodies. *Histochem Cell Biol* 112:457–465.
Sweadner KJ (1995) Na,K-ATPase and its isoforms. In: Neuroglia (Kettenmann H, Ransom BR, eds), pp 259–272. New York: Oxford University Press.
Tada Y, Horio Y, Kurachi Y (1998) Inwardly rectifying K^+ channel in retinal Müller cells: comparison with the KAB-2/Kir4.1 channel expressed in HEK293T cells. *Jpn J Physiol* 48:71–80.
Takumi T, Ishii T, Horio Y, Morishige K, Takahashi N, Yamada M, Yamashita T, Kiyami H, Sohmiya K, Nakanishi S, Kurachi Y (1995) A novel ATP-dependent inward rectifier potassium channel expressed predominantly in glial cells. *J Biol Chem* 270:16339–16346.
Tucker SJ, Imbrici P, Salvatore L, D'Adamo MC, Pessia M (2000) pH dependence of the inwardly rectifying potassium channel, Kir5.1, and localization in renal tubular epithelia. *J Biol Chem* 275:16404–16407.
Vyskocil F, Kritz N, Bures J (1972) Potassium-selective microelectrodes used for measuring the extracellular brain potassium during spreading depression and anoxic depolarization in rats. *Brain Res* 39:255–259.
Wallraff A, Kohling R, Heinemann U, Theis M, Willecke K, Steinhauser C (2006) The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. *J Neurosci* 26:5438–5447.
Walz W (1992) Role of Na/K/Cl cotransport in astrocytes. *Can J Physiol Pharmacol* 70 (Suppl):S260–S262.
Walz W (2002) Chloride/anion channels in glial cell membranes. *Glia* 40:1–10.
Xu X, Karwoski C (1997) The origin of slow PIII in frog retina: current source density analysis in the eyecup and isolated retina. *Vis Neurosci* 14:827–833.
Zahs KR, Kofuji P, Meier C, Dermietzel R (2003) Connexin immunoreactivity in glial cells of the rat retina. *J Comp Neurol* 455:531–546.
Zhou M, Schools GP, Kimelberg HK (2006) Development of GLAST(+) astrocytes and NG2(+) glia in rat hippocampus CA1: mature astrocytes are electrophysiologically passive. *J Neurophysiol* 95:134–143.

Abbreviations

AQP4	Aquaporin 4
CNS	Central nervous system
Cx	Connexins
DGC	Dystrophin glycoprotein complex
IOS	Intrinsic optic signal
KO	Knockout
$[K^+]_o$	Extracellular K^+ concentration
Kir channel	Inwardly rectifying K^+ channel
Na^+ pump	Na^+, K^+-ATPase