**Kir Potassium Channel Subunit Expression in Retinal Glial Cells: Implications for Spatial Potassium Buffering**

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**KEY WORDS** Müller cell; glia; retina; inwardly rectifying potassium channel; potassium siphoning

**ABSTRACT** To understand the role of different K⁺ channel subtypes in glial cell-mediated spatial buffering of extracellular K⁺, immunohistochemical localization of inwardly rectifying K⁺ channel subunits (Kir2.1, Kir2.2, Kir2.3, Kir4.1, and Kir5.1) was performed in the retina of the mouse. Stainings were found for the weakly inward-rectifying K⁺ channel subunit Kir4.1 and for the strongly inward-rectifying K⁺ channel subunit Kir2.1. The most prominent labeling of the Kir4.1 protein was found in the endfoot membranes of Müller glial cells facing the vitreous body and surrounding retinal blood vessels. Discrete punctate label was observed throughout all retinal layers and at the outer limiting membrane. By contrast, Kir2.1 immunoreactivity was located predominantly in the membrane domains of Müller cells that contact retinal neurons, i.e., along the two stem processes, over the soma, and in the side branches extending into the synaptic layers. The results suggest a model in which the glial cell-mediated transport of extracellular K⁺ away from excited neurons is mediated by the cooperation of different Kir channel subtypes. Weakly rectifying Kir channels (Kir4.1) are expressed predominantly in membrane domains where K⁺ currents leave the glial cells and enter extracellular “sinks,” whereas K⁺ influxes from neuronal “sources” into glial cells are mediated mainly by strongly rectifying Kir channels (Kir 2.1). The expression of strongly rectifying Kir channels along the “cables” for spatial buffering currents may prevent an unwarranted outward leak of K⁺, and, thus, avoid disturbances of neuronal information processing. GLIA 39:292–303, 2002.

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**INTRODUCTION**

One of the key roles of glial cells is the spatial buffering of extracellular K⁺ ions released by excited neurons especially at synaptic sites (Orkand et al., 1966; Kuffler et al., 1966; Chen and Nicholson, 2000). Glial cells transport K⁺ through their cell bodies away from excited neurons, from extracellular regions of high K⁺ (“sources”) to those of low K⁺ (“sinks”). During illumination of the retina, Müller glial cells are thought to mediate K⁺ fluxes from the two synaptic (plexiform) layers to three different sinks, i.e., the vitreous body, the blood vessels, and the subretinal space (Newman, 1984; Newman and Reichenbach, 1996), a process termed K⁺ siphoning (Newman, 1984). Generally, glial K⁺ buffering may be mediated by at least three mechanisms by (1) the Na⁺,K⁺-ATPase...
Fig. 1. Expression of various Kir channel subunits in murine retina. Subunit-specific anti Kir antibodies detect the expression of Kir2.1 (A–C) and Kir4.1 (J–L) in murine retinal slices, whereas no label is detected for Kir2.2 (D–F), Kir2.3 (G–I), and Kir5.1 (M–O). An anti-glutamine synthetase (GS) antibody was used to label the Müller cells (middle vertical row); the merge of Kir (green) and GS (red) immunoreactivities is shown in the right vertical column. IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar = 10 μm.
activity, (2) passive co-transport of K\(^+\) with other ions, and (3) K\(^+\) flux through open inwardly rectifying K\(^+\) (Kir) channels (Walz, 2000; Chen and Nicholson, 2000). However, although the Na\(^+\)/K\(^+\) pump and the K\(^+\) transporters redistribute extracellular K\(^+\) in a rather slow time course, fast K\(^+\) siphoning is thought to be mainly mediated by passive K\(^+\) fluxes through open Kir channels (Jauch et al., 1999). In particular, one subtype of Kir channels—the weakly rectifying Kir4.1 channel—has been implicated in spatial buffering of K\(^+\) by glial cells. This assumption is supported by electrophysiological data obtained for Müller cells from Kir4.1 knockout mice (Kofuji et al., 2000), as well as by investigations of the subcellular localization of the Kir4.1 channel protein in glial cells. In various brain regions, Kir4.1 immunoreactivity was found to be expressed in about one-half of the astrocytes, but not in neurons (Takumi et al., 1995; Poopalasundaram et al., 2000). In astrocytes of the olfactory bulb, Kir4.1 immunoreactivity was found at membrane sites abutting the blood vessels and the extracellular space, i.e., the regions in which K\(^+\) ions flow into Müller cells during K\(^+\) siphoning. In the present report, we show that Müller glial cells express at least two Kir channel subtypes that differ by the strength of their inward rectification, and that these different subtypes are located in distinct membrane regions. This prompted us to propose a modified model of K\(^+\) siphoning allowing for an optimized collaboration of specialized Kir channel subtypes.

**MATERIALS AND METHODS**

RT-PCR Analysis

The total RNA from adult murine retina was extracted using the RNA-Clean kit (Thermo Hybaid, Ashford, UK) and treated with DNase I (RNase-free from Stratagene, Amsterdam, The Netherlands) to prevent contamination by genomic DNA. cDNAs were synthesized by priming with oligo-dT and using Thermoscript Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). Polymerase chain reaction (PCR) was performed using the following primer pairs: Kir2.1, forward 5’-TTGGCTGTTGTTTTTGGTGTGAT-3’ and reverse 5’-AGTCTGGTTCATGTCTCTG-3’ (expected size 578 bp); Kir4.1, forward 5’-CCTCATTTGCTGCCAGGTGACA-3’ and reverse 5’-GTCCTTCTTTTTCAGCTTGCTC-3’ (487 bp); vimentin, forward 5’-TCAACACCGAGTTCAGAAAC-3’ and reverse 5’-GTTGTCGACCCACACTTTCA-3’ (575 bp); glutamine synthetase, forward 5’-GTGGAGAAAGTCGCTCTCC-3’ and reverse 5’-TTAGTTTCTCGATGGCCTCTC-3’ (766 bp). PCR amplification was performed for 40 cycles using Platinum Taq polymerase (Invitrogen) at 2 mM MgCl\(_2\). Each cycle consisted of 40 s at 94°C, 40 s at 54°C, and 1 min at 72°C. The amplified samples were analyzed by standard agarose gel electrophoresis.

**Kir4.1null (Kir4.1−/−) Mouse Line**

Mice with inactivation of the Kir4.1 gene (KCNJ10) are described in detail elsewhere (Kofuji et al., 2000). Retinae from Kir4.1−/− mice were removed from P18 to P20 animals and used for immunocytochemistry as described below. The genotype of the animals was confirmed by PCR as described previously (Kofuji et al., 2000).

**Immunohistochemical Analysis**

The following primary antibodies were used: a polyclonal rabbit anti-Kir4.1 (1:400; Alomone Laboratories, Jerusalem, Israel), a polyclonal rabbit anti-human Kir2.1 (1:400; Alomone Laboratories), a polyclonal chicken anti-mouse Kir2.2 (affinity-purified antibody was used at 1:100 dilution), a polyclonal rabbit anti-mouse Kir2.3 (1:400, Alomone Laboratories), a polyclonal anti-glutamine synthetase (GS) was detected.
clonal rabbit anti-mouse Kir5.1 (affinity-purified antibody was used at 1:50 dilution), mouse anti-vimentin antibody (1:1,000; V9 clone, Sigma, Deisenhofen, Germany), and a mouse anti-glutamine synthetase antibody (1:1,000; Chemicon, Temecula, CA). The secondary antibodies were a Cy3-conjugated goat anti-rabbit IgG (1:400; Dianova, Hamburg, Germany), a Cy2-conjugated goat anti-mouse IgG antibody (1:400; Dianova), and a FITC-conjugated goat anti-chicken IgG antibody (1:400, Jackson ImmunoResearch, West Grove, PA).

The anti Kir2.2 antibody was generated using a synthetic peptide corresponding to residues 356–413 TDRDVRTQPQEPHDLRILQAS as antigen. A reactive synthetic peptide to facilitate its conjugation to keyhole limpet hemocyanin carrier. Affinity purification of the antisera was performed with a column with immobilized Kir2.2 peptide. Bound anti-Kir2.2 antibody was eluted with 100 mM glycine, pH 2.5, and subsequently dialyzed against phosphate-buffered saline (PBS). The anti-Kir5.1 antibody was generated using a synthetic peptide corresponding to residues 394–413 KTSPARGSCNSDTNTRRR as antigen. Affinity purification of the antisera was performed using a column with immobilized Kir5.1 peptide. Bound anti-Kir5.1 antibody was eluted with 100 mM glycine, pH 2.5, and subsequently dialyzed against PBS.

Adult mice were deeply anesthetized by urethane (2 g/kg, i.p.) before decapitation and enucleation of the eyeballs. To label retinal sections, isolated retinai were fixed in 4% paraformaldehyde at 4°C for 30 min. After washing in PBS, the tissue was embedded in polyethylene glycol (PEG) 1450 (Sigma); 10-μm sections were made with a microtome and were mounted on gelatin-coated slides. To obtain single isolated cells, retinai were incubated with 0.4 mg/ml papain (Boehringer, Mannheim, Germany) in PBS (Seromed Biochrom, Berlin, Germany) at 37°C for 30 min. After fixation with 4% paraformaldehyde at 4°C for 10 min, the retinai were washed in PBS, and mechanically dissociated by trituration through a wide-pore pipette. Retinal sections and cells were washed in PBS before immunohistochemical processing. After blocking of the unspecific binding of the secondary antibody with normal goat serum (10%; Dianova, Hamburg, Germany) and permeabilization with 0.3% Triton X-100 in PBS for 1 h, the sections and cells were incubated in primary antibody at 4°C for 12 h. After washing in PBS, the secondary antibodies were applied for 2 h at room temperature. The labeling was visualized by means of a confocal laser scanning microscope LSM 510 (Zeiss, Oberkochen, Germany).

For the retinal whole mounts, the retinai were isolated and fixed in a 4% paraformaldehyde, 1 M phosphate solution, pH 7.4, overnight at 4°C. After washes with PBS, the retinai were blocked and permeabilized for 2 h in PBS solution containing 10% donkey serum and 1% Triton-X 100. Whole mounts were incubated with primary antibodies for 72 h at 4°C, followed by washes and incubation with secondary antibodies, FITC-conjugated donkey anti-rabbit IgG (1:400; Jackson ImmunoResearch, West Grove, PA) and TRITC conjugated donkey anti-mouse IgG (Jackson ImmunoResearch) for 72 h at 4°C. Optical sections were collected at 0.75–1.5-μm intervals. Data are presented as projections of several optical images onto a single plane.

RESULTS

Kir Channel Subunits in Mouse Retina

Earlier studies have determined the expression pattern of Kir4.1 in the mouse and rat retina (Ishii et al., 1997; Nagelhus et al., 1999; Kofuji et al., 2000), rat cerebellum and hippocampus (Stonehouse et al., 1999; Poopalasundaram et al., 2000), and mouse cortical astrocytes (Li et al., 2001). Herein, we investigated the cellular and subcellular distribution of constitutively active inward rectifiers Kir 2.1, 2.2, 2.3, 4.1, and 5.1 in murine retinae, using subunit-specific anti-Kir antibodies.

Whereas Kir2.2, Kir2.3, and Kir5.1 immunoreactivities could not be demonstrated in the mouse retina (Fig. 1), Kir2.1 and Kir4.1 immunostaining were readily apparent (Fig. 1). This confirms earlier data on retinal Kir4.1 expression (Ishii et al., 1997; Nagelhus et al., 1999; Kofuji et al., 2000). Furthermore, co-immunostaining with glutamine synthetase (GS), a frequently used marker for Müller cells (Kofuji et al., 2000), showed some degree of overlap of GS and Kir2.1 (Fig 1C), suggesting the additional expression of Kir2.1 subunits in Müller cells (see also below).

It is unlikely that the lack of signal for Kir2.2, Kir2.3, and Kir5.1 arises from a low immunoreactivity of the antibodies used. Indeed, the anti Kir 2.2 and 5.1 antibodies showed strong immunoreactivity in cells transfected with Kir2.2 and Kir5.1 subunit cDNAs, but not in those cells transfected with Kir2.1, Kir4.1, and Kir3.1 subunit cDNAs (data not shown). Moreover, the anti-Kir2.2 antibody when used to immunostain paraffin sections of mouse cerebellum showed strong labeling of cells in the molecular and granule cell layer as expected from in situ hybridization studies (Karschin et al., 1996) and from a previous immunohistochemical study in rat cerebellum (Stonehouse et al., 1999). Likewise, the anti-Kir 5.1 antibody showed strong immunoreactivity against basolateral cells in tubules of rat kidney, and the anti-Kir 2.3 antibody was also highly immunoreactive in COS-7 cells transfected with Kir2.3 subunit (data not shown).

In the RT-PCR analysis of total RNA extracted from murine retinai, the oligonucleotide primers for Kir2.1 and Kir4.1 showed amplification of products of expected sizes (Fig. 2). As a positive control, mRNAs for vimentin (vim) and for GS were found at the expected sizes. To demonstrate the cellular expression of the two Kir channel subunit proteins, immunohistochemical studies were done.
Kir4.1 Immunoreactivity

Figure 3A,C,E shows in more detail the pattern of distribution of Kir4.1 immunoreactivity in a mouse retina. The staining of the Kir4.1 protein was most prominent at the inner limiting membrane, i.e. in the endfoot membranes of Müller cells abutting the vitreous body (arrow in Fig. 3A), and around the blood vessels in the inner nuclear layer, where Müller cells form “en-passant-endfeet” (arrow in Fig. 3C). Less prominent staining was found near the outer limiting membrane, probably in the microvilli of Müller cells that extend into the subretinal space (arrow in Fig. 3E; cf. Nagelhus et al., 1999). In addition, scattered clusters of Kir4.1 immunostaining were observed throughout all retinal layers. Double immunostaining of Kir4.1 and vimentin showed that the clusters of Kir4.1 immunoreactivity were mainly colocalized with vimentin-positive radially oriented cell processes, suggesting an expression of Kir4.1 protein on Müller cells (not

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Fig. 4. Immunoreactivity of the Kir2.1 channel subunit (left vertical column; green) and glutamine synthetase (GS; middle vertical column; red) in the murine whole mount retina (P21). The two immunoreactivities are merged in the right vertical column. Confocal optical images were obtained at the level of the nerve fiber layer (NFL) (A–C), inner plexiform layer (IPL) (D–F), inner nuclear layer (INL) (G–I), outer plexiform layer (OPL) (J–L) and outer nuclear layer (ONL) (M–O). Scale bar = 30 μm.
A virtually identical retinal localization of the Kir4.1 immunoreactivity was observed in rat retinas (not shown). As described previously (Kofuji et al., 2000), Kir4.1 labeling was absent in somata and proximal processes of astrocytes which are located in the superficial nerve fiber layer of murine (Figs. 1J–L, 3A) and rat retinas (not shown).

To verify the location of Kir4.1 immunoreactivity on Müller cells, cells were proteolytically and mechanically isolated from murine retinae. In isolated Müller cells, Kir4.1 immunolabeling was observed predominantly in the apical endfoot area, with significantly weaker labeling over the other membrane domains (see Fig. 5A,B). This expression pattern is very similar to that observed in retinal slices, supporting the view that the Kir4.1 channel protein is predominantly or even exclusively expressed by Müller cells. The data also indicate that the Kir4.1 channel protein is accumulated selectively in such Müller cell membrane areas, which contact two structures considered as main current sinks for retinal K⁺ siphoning, i.e., the vitreous body and the blood vessels.

### Kir2.1 Immunoreactivity

Intense Kir2.1 immunoreactivity was visible throughout the thickness of murine retinas (Fig. 3B,D,F), especially along radially oriented cellular elements in the inner plexiform layer, between the apparently nonstained ganglion cell somata, and around cell bodies in the inner nuclear layer; in the outer nuclear layer, honeycomb-like structures were immunopositive for Kir2.1. Although neither ganglion cell nor photoreceptor cell somata showed distinct expression of Kir2.1 immunoreactivity, both were enveloped by Kir2.1 staining, which indicates that the lateral membranes of Müller cell endfeet in the ganglion cell layer, as well as their honeycomb-meshwork sheaths in the outer nuclear layer, express Kir2.1 protein. In contrast to the case of Kir4.1 protein expression, the inner limiting membrane was only weakly stained with Kir2.1 antibodies indicating that there is low—if any—expression of Kir2.1 protein in the membranes of Müller cell endfeet abutting the vitreous body.

The inner nuclear layer contains the somata of Müller cells and of second-order neurons such as bipolar and amacrine cells. Usually, the somata of bipolar and amacrine cells display an almost circular shape in retinal wild-type mice (see Figs. 1 and 3). Overlay of confocal images with the Müller cell marker glutamine synthetase (GS; red) shows the expression of Kir2.1 in Müller cells. Scale bar, 10 μm.
inal sections whereas the somata of Müller cells appear more “angular” as if intended by the somata of neighboring neurons (e.g., Grosche et al., 1997). In the inner nuclear layer of the murine retina, strong Kir2.1 immunoreactivity was observed on the angular somata of Müller cells, whereas the round somata of neurons showed weak or no staining (Figs. 3D and 4G–I). Furthermore, the virtually perfect co-localization of Kir2.1 and GS immunoreactivities throughout the various retinal layers as revealed by confocal microscopy of retinal whole mounts (Fig. 4) indicates that both the vitread and the sclerad Müller cell processes display strong Kir2.1 immunolabel in their membranes.

To confirm that Müller cells express Kir2.1 immunoreactivity, isolated Müller cells were stained. Indeed, Kir2.1 immunolabeling was observed in large membrane areas including the endfeet, the soma and the two stem processes of the cells (Fig. 5C,D).

**Kir Channel Immunoreactivity in Kir4.1 null (Kir4.1−/−) Mouse Retina**

As previously described (Kofuji et al., 2000), the retina of Kir4.1 knockout mice lacks mRNA for Kir4.1 channel subunits, while mRNAs for other subunits, including Kir2.1, are still expressed. To verify the expression of Kir2.1 in retinas from Kir4.1 knockout mouse, retinas from these mutant mice were stained with anti-Kir2.1 antibody. As shown in Figure 6, Kir2.1 immunoreactivity was present in retinal sections from Kir4.1 knockout mice in a pattern very similar to that described for the wild-type (cf. Figs. 1 and 3).

**Developmental Expression Pattern of Kir Channels in Mouse Retina**

We also have performed the immunocytochemical localization of Kir2.1 and Kir4.1 in postnatal murine retinae (postnatal days = P9, P12, P14, P16, P24, and P71). Kir 2.1 and 4.1 immunoreactivities were more prominent from P16 (Fig. 7), when the pattern of immunoreactivity for Kir2.1 and Kir4.1 described for the adult murine retinas was clearly seen. At P24 and P71, the Kir2.1 and Kir4.1 immunoreactivities seem to be qualitatively similar albeit of stronger intensity. Immunoreactivity for the Müller cell marker glutamine synthetase was apparent from P12 day (data not shown). Thus, Kir2.1 and Kir4.1 in murine retinas shows a pattern of overlapping and coordinated temporal expression, suggestive of a common or complementary role in murine retinas.

**DISCUSSION**

Using different primers, we confirm the expression of mRNA (Kofuji et al., 2000) and demonstrate the expression of immunoreactivity for two subtypes of Kir channel subunits in the retina of the mouse. Both subtypes are present in Müller cells, whereas their expression by retinal neurons is significantly less pronounced, or even absent. The subtypes differ in their physiological properties (cf. “idealized” current–voltage characteristics in Fig. 8). Kir2.1 channels are strongly rectifying Kir channels which allow inward K⁺ currents but almost no outward K⁺ currents even at very high extracellular K⁺ concentrations (Yang et al., 2000). By contrast, Kir4.1 channels (Shuck et al., 1997) are very weakly rectifying Kir channels at negative potentials, allowing either inward or outward K⁺ currents in dependence on the membrane potential and on the concentration of extracellular K⁺. The currents through Kir4.1 channels show a linear dependence on the extracellular K⁺ concentration (Shuck et al., 1997), while the K⁺ dependence of Kir2.1 channel currents is exponential; i.e., the inwardly directed potassium currents increase exponentially when the extracellular K⁺ concentration rises (Yang et al., 2000).
Kir4.1 channels apparently fail to affect the expression of Kir2.1 immunoreactivity (Fig. 6) and mRNA (Kofuji et al., 2000) by Müller cells.

**Kir Channels and Müller Cell Electrophysiology**

If Müller cells indeed express more than one subtype of functional Kir channel subunit, how does this fit with electrophysiological observations reported in the literature? Recently, Müller cells from Kir4.1 knockout mice were studied (Kofuji et al., 2000). As compared with control cells, these cells showed a strongly increased input resistance and a very depolarized resting membrane potential, although the expression of Kir2.1 mRNA (Kofuji et al., 2000) and protein (Fig. 6) was apparently unchanged. Although it is possible that knocking out the Kir4.1 channels in Müller cells may decrease the availability of Kir2.1 channels via some (unknown) biochemical mechanisms, the findings reported by Kofuji and coworkers (2000) may also be explained by the fact that only weakly rectifying channels (e.g., the Kir4.1 subunit) are appropriate to generate a negative membrane potential during the course of Müller cell differentiation from depolarized progenitor cells (cf. Bringmann et al., 2000) and to restore such a potential in mature cells after depolarization that frequently occur in response to neuronal activity. By contrast, the strongly rectifying Kir2.1 channels are activated only at very negative potentials in physiological extracellular K⁺ (Yang et al., 2000). Thus, in the Kir4.1 knockout mice, the Müller cells never develop the typical negative resting membrane potential, and the Kir2.1 channels—even if present and unblocked—can neither open nor contribute to the membrane conductance of the cells.

**Kir Channels and K⁺ Siphoning**

Kir channels of Müller cells are thought to mediate the buffering of the extracellular K⁺ in dependence on the neuronal activity (Newman, 1985, 1993; Brew et al., 1986; Reichenbach et al., 1992; Newman and Reichenbach, 1996). In particular, the Kir4.1 channel subunit has been implicated in Müller cell-mediated spatial buffering of K⁺ (Kofuji et al., 2000). In Müller cells, the Kir4.1 channel subunit expression is focussed in membrane domains which contact extracellular compartments serving as sinks for K⁺ siphoning currents (i.e., mediating K⁺ efflux from Müller cells; Fig. 7. Immunoreactivity of Kir2.1 and Kir4.1 in wild-type murine retinae at the postnatal developmental days 9, 12, 14, 16, 24, and 71. Note the Kir 2.1 expression from P14 in the cell soma, and from P24 in the entire Müller cells including cell body, processes and endfeet. Kir 4.1 is also expressed on P14 at the vitread Müller cell endfeet; thereafter, it is expressed around the blood vessels, and, less prominent, on Müller cell somata and processes. GCL, ganglion cell layer, INL, inner nuclear layer, ONL, outer nuclear layer. Scale bar = 30μm.)
Fig. 8. Semi-schematic drawing of the distribution of Kir channel subtypes in Müller cells, and their contribution to retinal K⁺ siphoning. A: Classical view (Newman, 1985; Reichenbach et al., 1992). Only weakly rectifying Kir4.1 channels (red) are expressed, with high protein densities only in the vitread and perivascular membranes. During retinal illumination, the extracellular K⁺ concentration is elevated particularly in the two synaptic layers, the inner (IPL) and outer plexiform layers (OPL). This causes a K⁺ influx into Müller cells, and a simultaneous K⁺ release into the vitreous humor, the blood vessels, and the subretinal space. The small simplified circuit (top right) exemplifies only a part of this mechanism, the siphoning from the source in the IPL into the vitreous body as the sink, and the backflow through the extracellular space (ECS; green arrow). As the Kir4.1 channels are rare in the membrane facing the source (pink color; small arrows at the right side of the cell) and allow for both inflow and outflow of K⁺ (bidirectional arrows), the potassium influx is rather small, and shunting outward currents may occur in the circuit; the K⁺ efflux across the endfoot membrane is smaller than the local conductance would allow (gray arrow). B: Novel model, based upon the presence and specific localization of two different Kir channel subunits (Kir4.1; red; Kir2.1, blue). The weakly rectifying Kir channels (Kir4.1; bidirectional arrows) are expressed predominantly in membranes through which K⁺ ions flow out of Müller cells during light-evoked retinal activity. The strongly rectifying Kir2.1 channel subtype (unidirectional arrows) dominates the membranes which have contact to the perineuronal spaces (the K⁺ sources) and which mediate the K⁺ influx. The exemplary circuit (top right) is now endowed with sufficient inward conductance at the source-faced areas, and lacks shunt currents; thus, the outward conductance can be used to full extend (black arrow). At bottom right of A and B, idealized current–voltage relationships of the two Kir channel subunits are shown as taken from the literature (see text). While Kir4.1 channels are open over a wide range of membrane potentials, and allow for inward and outward currents, Kir2.1 channels can open only at very negative membrane potentials and are virtually perfect inward rectifiers.

8A), whereas only weak Kir4.1 expression is found in front of the K⁺ sources where the influx occurs (Ishii et al., 1997; Nagelhus et al., 1999; Kofuji et al., 2000; Poopalsundaram et al., 2000). However, the total current in a circuit is dependent on the highest resistance in series. This prompted a long-lasting discussion pointing out that from a teleological point of view, equal input and output resistances would allow for a more efficient K⁺ siphoning than the observed asymmetrical distribution of the Kir4.1-mediated conductance (Brew and Attwell, 1985; Chen and Nicholson, 2000). In the present study, we show that, in addition to Kir4.1, Kir2.1 subunits are expressed in Müller cells, suggesting that retinal K⁺ siphoning may be mediated by the cooperation of different Kir channel subtypes. This should greatly improve the efficacy of spatial buffering currents, as shown in Figure 8B. The classical view (Fig. 8A) is that Müller cells are endowed only with Kir4.1 channels which are distributed in a highly asymmetrical manner. This makes the entire cell membrane permeable for K⁺ fluxes in both directions (inward and outward) but a high conductance is only displayed by the membrane areas facing the extracellular sinks. Assuming, for simplification, that the [K⁺]ₑ increases only in the IPL and must be buffered only into the vitreous body, the small circuit (right upper part of Fig. 8A) demonstrates two problems. First, the conductance in the source-facing membrane is too small, and second, shunting outward currents may occur before the main intracellular current arrives at the large endfoot conductance. Thus, much of the latter cannot be used (the outward arrow is drawn in gray), and the current remains smaller than it could be. If, however, additional Kir2.1 channels are inserted in large parts of the membrane (Fig. 8B), the K⁺ influx in the source areas is improved, and shunt currents are avoided. Thus, the large efflux conductance of the endfoot membrane can be used to full extend (large black arrow in the circuit), and K⁺ siphoning is optimized. By analogy, the transport of K⁺ ions to the other sinks (the retinal blood vessels and the subretinal space) should also be improved.

This model is compatible with recent observations in Müller cells from Kir4.1 knockout mice (Kofuji et al., 2000), in which the slow PIII wave of the electroretinogram, assumed to be generated by K⁺ fluxes through
Müller cells, is abolished despite of unchanged expression of Kir2.1. In this case, blocking out of the Kir4.1 channels will not only disrupt the current loops underlying the K⁺ siphoning mechanism (cf. Fig. 8) but also prevent an opening of the Kir2.1 channels because the membrane potential is too depolarized for an activation of these channels (see above section).

A similar situation exists in regard to brain astrocytes; only subpopulations, in distinct brain regions, express Kir4.1 channel subunits (Takumi et al., 1995; Poopalasundaram et al., 2000; Higashi et al., 2001; Schröder et al., 2002) thought to be implicated in spatial buffering of K⁺ (Higashi et al., 2001). This diversity may be reflected by a wide variety of resting membrane potentials, e.g., in rat hippocampal astrocytes (McKhan et al., 1997). The expression of Kir2.1-2.3 channel mRNA in mice brain has been described (Kofuji et al., 2000). Using single-cell RT-PCR, the expression of mRNA encoding Kir2.1-2.3 was recently described in CA1 stratum radiatum astrocytes in murine brain (Schröder et al., 2002). Astrocytes in rat brain express Kir2.1-2.3 protein in dependence on the brain region (Stonehouse et al., 1999; Leonoudakis et al., 2001). It is well conceivable that in brain as well as in the retina, a cooperation of several subtypes of Kir channels may be involved in spatial buffering. In particular, these different Kir channels may be specifically distributed among different groups or even individuals of astrocytes, as the members of the gap junction-coupled astrocytic network may perform a “division of labor,” which in Müller cells is realized by specialized membrane areas.

In summary, the present results indicate that in Müller cells, (at least) two distinct types of Kir channel subunits are specifically distributed over cell membrane areas with different environmental relationships and with different functions in K⁺ siphoning. Strongly rectifying Kir2.1 channels may mediate the K⁺ influx currents from retinal neurons into Müller cells while weakly rectifying Kir4.1 channels mediate the K⁺ efflux from Müller cells into extracellular K⁺ buffering sinks. This arrangement accelerates the K⁺ clearance and directs the underlying currents away from neuronal cells, constituting a mechanism that avoids any affection of neuronal information processing by depolarization caused by glia-derived K⁺.

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REFERENCES


Brew H, Gray PTA, Mobbs P, Attwell D. 1986. Vap4 of retinal glial cells may be re-

REFERENCES


