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Proliferative gliosis causes mislocation and inactivation of inwardly rectifying K^+ (Kir) channels in rabbit retinal glial cells

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Abstract

Retinal glial (Müller) cells are proposed to mediate retinal potassium homeostasis predominantly by potassium transport through inwardly rectifying K^+ (Kir) channels. Retinal gliosis is often associated with a decrease in glial potassium conductance. To determine whether this decrease is caused by a downregulation of glial Kir channels, we investigated a rabbit model of proliferative vitreoretinopathy (PVR) which is known to be associated with proliferative gliosis. The membrane conductance of control Müller cells is characterized by large Kir currents whereas Müller cells of PVR retinas displayed an almost total absence of Kir currents. In control tissues, Kir2.1 immunoreactivity is localized in the inner stem processes and endfeet of Müller cells whereas Kir4.1 immunoreactivity is largely confined to the Müller cell endfeet. In PVR retinas, there is a mislocation of Kir channel proteins, with Kir4.1 immunoreactivity detectable in Müller cell fibers throughout the whole retina, and a decrease of immunoreactivity in the cellular endfeet. Real-time PCR analysis revealed no alteration of the Kir4.1 mRNA levels in PVR retinas as compared to the controls but a slight decrease in Kir2.1 mRNA. Western blotting showed no difference in the Kir4.1 protein content between control and PVR retinas. The data suggest that proliferative gliosis in the retina is associated with a functional inactivation of glial Kir channels that is not caused by a downregulation of the channel proteins but is associated with their mislocation in the cell membrane.

Keywords: Kir4.1; Kir2.1; potassium current; gliosis; retina

1. Introduction

Glial cells play a major role in the ionic and osmotic homeostasis of the central nervous system. Neuronal activity causes local elevations in extracellular potassium which are buffered by glial cells. In the neural retina, Müller glial cells are suggested to take up excess potassium from the synaptic layers, and to release potassium ions into the blood and vitreous (Bringmann et al., 2006; Newman and Reichenbach, 1996). The transglial potassium transport is facilitated by inwardly rectifying potassium (Kir) channels expressed in the plasma membranes (Newman, 1993), and supported by a concomitant water flux through aquaporin water channels

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(Nagelhus et al., 1999). Among the various Kir channel subunits expressed by Müller cells (Raap et al., 2002), especially Kir4.1 and Kir2.1 channels have been implicated in mediating the potassium buffering currents (Kofuji et al., 2000, 2002).

Both the potassium conductance and the Kir channels are unevenly distributed along the plasma membrane of Müller cells. The membrane conductance is high in subcellular domains facing extra-retinal fluid-filled spaces, i.e., the microvasculature and the vitreous body (Newman, 1987). The high potassium conductance of these cellular compartments has been explained by a high density of Kir4.1 channels localized in these membrane domains (Connors and Kofuji, 2002; Nagelhus et al., 1999). In contrast to Müller cells of vascularized retinas, Müller cells of avascular retinas such as of the rabbit and guinea pig, display the most prominent potassium conductance in the membranes of the cellular endfeet in the innermost retinal layers, facing the vitreous body (Newman, 1987). However, it is unknown whether this strongly polarized distribution of the potassium conductance in Müller cells of avascular retinas is also related to an asymmetric localization of Kir4.1 channels.

Reactive gliosis in the retina is often associated with a decrease in the potassium conductance and a mislocation and/or downregulation of Kir4.1 channels in Müller cells (Francke et al., 2001; Pannicke et al., 2004, 2005, 2006). Until today, it is unclear whether the decrease in the potassium conductance of rabbit Müller cells is caused by a downregulation of Kir4.1. A downregulation or inactivation of Kir channels has been proposed to represent a hallmark of glial cellular dedifferentiation, and to be a precondition for Müller cell proliferation (Bringmann et al., 2000). Proliferative gliosis is associated with an almost complete absence of Kir currents in human Müller cells (Bringmann et al., 1999; Francke et al., 1997). Proliferative retinopathies (proliferative vitreoretinopathy, PVR) are a serious blinding complication of retinal detachment and can also be evoked by retinal surgery. PVR is characterized by the uncontrolled tumor-like proliferation of various cell types including glial cells, and results in the formation of periretinal contractile fibrocellular membranes that cause secondary tractional retinal detachment. Retinal detachment leads to photoreceptor degeneration and reactive changes in the inner retinal neurons (Fisher and Lewis, 2003). Here, we used a rabbit model of PVR to determine whether proliferative gliosis in the retina is associated with a mislocation and/or downregulation of Müller glial Kir channels.

2. Materials and methods

2.1. Animal model of PVR

All experiments were done in accordance with the European Communities Council Directive 86/609/EEC, and were approved by the local authorities. Eight adult pigmented rabbits (2.5–3.5 kg; both sexes) were used. The animals were held under 12 h:12 h light/dark (day/night) room conditions, with free access to food and water. According to a method described previously (Francke et al., 2002), PVR was induced in one eye of the animals while the other eye remained untreated and served as control. Anesthesia was induced by intramuscular ketamine (50 mg/kg) and xylazine (3 mg/kg; BayerVital, Leverkusen, Germany). The pupils of the eyes were dilated with topical tropicamide (1%; Ursapharm, Saarbrücken, Germany) and phenylephrine (5%; Ankerpharm, Rudolstadt, Germany). After pars plana sclerotomy, a circumscript vitrectomy was performed in the area of the future retinal detachment (in the ventro-nasal quadrant, below the medullary rays). A thin glass micropipette attached to a 250-µl Hamilton glass syringe was used to create a small local retinal detachment by injecting phosphate-buffered saline (pH 7.4) into the subretinal space. Another micropipette placed in the vitreous near the surface of the detached retina was used to inject 100 µl saline containing the proteolytic enzyme, dispase I (0.5 U; Boehringer, Mannheim, Germany). After injections, the sclerotomies and the conjunctiva were closed. Two weeks after operation, the animals were anaesthetized as above, and killed by intravenous T61 (3 ml; embutramid 0.2 g/ml, mebezonium iodide 0.05 g/ml, tetracain hydrochloride 5 mg/ml; Hoechst, Unterschleißheim, Germany), and the eyes were removed.

2.2. Electrophysiological recordings

Pieces of retinal tissue were incubated in papain (0.2 mg/ml; Roche Molecular Biochemicals)-containing calcium- and magnesium-free phosphate-buffered saline (pH 7.4) for 30 min at 37 °C, followed by several washing steps with normal saline. After short incubation in saline supplemented with DNase I (200 U/ml; Sigma–Aldrich, Taufkirchen, Germany), the tissue pieces were triturated by a wide-pore pipette, to obtain suspensions of isolated cells. The cells were stored at 4 °C in serum-free minimum essential medium until use within 4 h after cell isolation.

Membrane currents of acutely isolated Müller cells were recorded in the whole-cell configuration of the patch-clamp technique. Voltage-clamp records were performed at room temperature (22 °C) using the Axopatch 200A amplifier (Axon Instruments, Foster City, CA, USA) and the ISO-2 software (MFK, Niedernhausen, Germany). The signals were low-pass filtered at 1 kHz (eight-pole Bessel filter) and digitized at 5 kHz, using a 12-bit A/D converter. Patch pipettes were pulled from borosilicate glass (Science Products, Hofheim, Germany) and had resistances between 4 and 6 MΩ when filled with the intracellular solution containing (in mM) 10 NaCl, 130 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 ethyleneglycolbis(aminoethylether)tetra-acetate, and 10 *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid (HEPES) adjusted to pH 7.1 with Tris-base. The recording chamber was continuously perfused with extracellular solution which contained (in mM) 135 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1 Na_2HPO_4, 10 HEPES-Tris, and 11 glucose (pH 7.4). To evoke potassium currents, depolarizing and hyperpolarizing voltage steps of 250 ms duration, with increments of 10 mV, were applied from a holding potential of −80 mV. The membrane capacitance of the cells was measured by the integral of the uncompensated capacitive artifact (filtered at 6 kHz) evoked by a hyperpolarizing voltage