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Abstract

Excessive light causes damage to photoreceptor and pigment epithelial cells, and a local edema in the outer retina. Since Müller glial cells normally mediate the osmohomeostasis in the inner retina (mainly via channel-mediated transport of potassium and water), we determined whether retinal light injury causes an alteration in the retinal localization of glial water (aquaporin-4) and potassium (Kir4.1) channels, and in the potassium conductance of Müller cells. Mice were treated with bright white light (intensity, 15,000 lux) for two hours. Light treatment results in Müller cell gliosis as indicated by the enhanced staining of the glial fibrillary acidic protein and an increase in the cell membrane area reflecting cellular hypertrophy. In light-injured retinas, the immunostaining of the photoreceptor water channel aquaporin-1 disappeared along with the degeneration of the outer retina, and the outer nuclear layer contained large spherical bodies representing photoreceptor nuclei which were fused together. The immunostainings of the aquaporin-4 and Kir4.1 proteins were increased in the outer retina after light treatment. Since the amplitude of the potassium currents of Müller cells remained largely unaltered, the increase in the Kir4.1 immunostaining is supposed to be caused by a redistribution of the channel protein. The data indicate that Müller glial cells respond to excessive light with an alteration in the localization of Kir4.1 and aquaporin-4 proteins; this alteration is thought to be a response to the edema in the outer retina and may support the resolution of edema.
Localization of glial aquaporin-4 and Kir4.1 in the light-injured murine retina

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Abstract

Excessive light causes damage to photoreceptor and pigment epithelial cells, and a local edema in the outer retina. Since Müller glial cells normally mediate the osmohomeostasis in the inner retina (mainly via channel-mediated transport of potassium and water), we determined whether retinal light injury causes an alteration in the retinal localization of glial water (aquaporin-4) and potassium (Kir4.1) channels, and in the potassium conductance of Müller cells. Mice were treated with bright white light (intensity, 15,000 lux) for two hours. Light treatment results in Müller cell gliosis as indicated by the enhanced staining of the glial fibrillary acidic protein and an increase in the cell membrane area reflecting cellular hypertrophy. In light-injured retinas, the immunostaining of the photoreceptor water channel aquaporin-1 disappeared along with the degeneration of the outer retina, and the outer nuclear layer contained large spherical bodies representing photoreceptor nuclei which were fused together. The immunostainings of the aquaporin-4 and Kir4.1 proteins were increased in the outer retina after light treatment. Since the amplitude of the potassium currents of Müller cells remained largely unaltered, the increase in the Kir4.1 immunostaining is supposed to be caused by a redistribution of the channel protein. The data indicate that Müller glial cells respond to excessive light with an alteration in the localization of Kir4.1 and aquaporin-4 proteins; this alteration is thought to be a response to the edema in the outer retina and may support the resolution of edema.

Keywords: Retina; Müller cell; Aquaporin-4; Kir4.1; Light injury; Mouse

Extensive illumination damages the retinal tissue. In particular, short wavelength light is responsible for solar retinitis, plays a role in the pathogenesis of age-related macular degeneration, and (as a component of the light of ophthalmological instruments) contributes
to the development of macular edema after surgery [17]. Excessive light causes death of photoreceptor cells and damage to the retinal pigment epithelium. The death of photoreceptor cells occurs predominantly via apoptosis [13,16]. The damage to the photoreceptor and pigment epithelial cells is accompanied by the development of a local edema in the outer retina, due to the breakdown of the outer blood-retinal barrier normally constituted by the pigment epithelium [12], and a normotonic shrinkage of the cells that undergo apoptosis. The volume decrease of apoptotic cells occurs via channel- and transporter-mediated efflux of osmolytes (especially of potassium, sodium, and chloride ions); the ion efflux creates an osmotic gradient that draws water out of the cells [1,7,18].

Normally, the osmohomeostasis in the retina is regulated by the pigment epithelium and Müller glial cells [2,3,11]. While the pigment epithelium dehydrates the subretinal space and the outer retina, Müller cells mediate the osmohomeostasis predominantly in the inner retina. The transcellular water transport is facilitated by water-selective channels, the aquaporins. Aquaporins mediate bidirectional water transport across membranes in response to osmotic gradients and differences in hydrostatic pressure [15]. The water transport through pigment epithelial and Müller cells is coupled to the transport of osmolytes, especially of potassium and chloride ions [9,11]. While the water transport within the outer retina is facilitated by aquaporin-1 channels expressed by photoreceptor and pigment epithelial cells [4,14], Müller cells express the glial water channel aquaporin-4 predominantly in the inner retina [8]. The co-localization of aquaporin-4 and the inwardly rectifying potassium channel Kir4.1 in distinct membrane domains of Müller cells has led to the suggestion that the rapid water and potassium fluxes through Müller cells are coupled [9]. Kir4.1 is the major channel involved in the spatial buffering of the retinal potassium concentration mediated by Müller cells [5]. After light injury, the edema in the outer retina can not be resolved by the damaged pigment epithelium and, thus, we assume that Müller glial cells are involved in the resolution of the outer retinal edema. Therefore, we investigated whether Müller cells respond to the light-evoked edema in the outer retina with alterations in the localization of aquaporin-4 and Kir4.1 channel proteins, and in their potassium conductance.

All procedures concerning animals were in accordance with the European Communities Council Directive 86/609/EEC. The animals were reared in 12 hours (6:00 AM -6:00 PM) light/dark cycles with 60-100 lux within the cages. Light damage in retinas of pigmented C57B/6–SV129 mice (age, 10 weeks) was induced by exposure to diffuse white fluorescent light for two hours (lights on at 10:00 AM). Before light exposure, all animals were dark-adapted for 16 hours overnight. The pupils of the animals were dilated under dim red light (Cyclogyl 1%; Alcon, Cham, Switzerland; and phenylephrine 5%, Ciba Vision, Niederwangen, Switzerland), and freely-moving mice were exposed to light (TLD-36 W/965 tubes; Philips, Hamburg, Germany; UV-impermeable diffuser) with an intensity of 15,000 lux in cages with a reflective interior. At various time periods after treatment, the animals were killed by carbon dioxide, and the eyes were removed.

Isolated retinas were fixed in 4% paraformaldehyde for two hours. After several washing steps in buffered saline, the tissues were embedded in saline containing 3% agarose (w/v), and 80-µm thick slices were cut by using a vibratome. The slices were incubated in 5% normal goat serum plus 0.3% Triton X-100 in saline for two hours at room temperature and, subsequently, in primary antibodies overnight at 4°C. After washing in 1% bovine serum albumin in saline, the secondary antibodies were applied for two hours at room temperature. The lack of unspecific staining was proven by negative controls omitting the primary antibodies (not shown). Images were recorded with a confocal laser scanning microscope at single planes; excitation and emission settings were held constant for all images acquired. The following antibodies were used: mouse anti-glutamine synthetase (1:250; Chemicon), mouse anti-glial fibrillary acidic protein (GFAP; 1:200; G-A-5 clone, Sigma), rabbit anti-Kir4.1 (1:200; Alomone Labs), rabbit anti-aquaporin-1 (1:200; Chemicon), rabbit anti-rat aquaporin-
Whole-cell patch-clamp recordings were carried out at room temperature (22-25°C) using Müller glial cells acutely isolated in papain and DNase I-containing solutions, as described previously [10]. The cell suspensions were stored in serum-free minimum essential medium (Sigma-Aldrich) at 4°C (up to 6 hours) before use. Voltage-clamp recordings were performed using the Axopatch 200A amplifier (Axon Instruments, Foster City, CA) and the ISO-2 computer program (MFK, Niedernhausen, Germany). 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 that contained (mM) 10 NaCl, 130 KCl, 1 CaCl₂, 2 MgCl₂, 10 EGTA, and 10 HEPES-Tris (pH 7.1). To assure that the present results are comparable with previously published data [10], spermine or adenosine 5’-triphosphate were not included in the intracellular solution. The signals were low-pass filtered at 1, 2, or 6 kHz (eight-pole Bessel filter) and digitized at 5, 10, or 30 kHz, respectively, using a 12-bit A/D converter. The recording chamber was continuously perfused with extracellular solution consisting of (mM) 135 NaCl, 3 KCl, 2 CaCl₂, 1 MgCl₂, 1 Na₂HPO₄, 10 HEPES, 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 step from -80 to -90 mV when barium chloride (1 mM) was present in the bath solution to block the potassium currents through Kir channels. The resting membrane potential was measured in the current-clamp mode. The amplitude of the inward currents of isolated cells was measured at the end of the 250-ms voltage step from -80 to -140 mV. Statistical analysis was made using SigmaPlot (SPSS Inc., Chicago, IL); significance was determined by Kruskal-Wallis test followed by Dunn's comparison. Data are expressed as means ± SD.

Treatment of mice with bright white light caused photoreceptor cell degeneration which was reflected by the disappearance of the photoreceptor inner and outer segments and a thinning of the outer nuclear layer (Fig. 1A). Photoreceptor cells express aquaporin-1 [4]; the aquaporin-1 staining disappeared along with the degeneration of the outer retina. After ten days of light exposure, aquaporin-1 surrounded only the few remaining photoreceptor nuclei (Fig. 1A). Interestingly, at all stages investigated after light exposure the outer nuclear layer contained (in addition to photoreceptor nuclei of normal size) large spherical bodies (Fig. 1A,C,D). The staining of these bodies with the DNA dye Hoechst 33258 suggests that these structures represent (apoptotic) photoreceptor nuclei which were fused together.

Treatment with bright white light induced an activation of Müller glial cells in retinas of mice, as indicated by the expression of the intermediate filament GFAP in Müller cell fibers. Whereas in control retinas, GFAP immunolabeling is restricted to the astrocytes in the ganglion cell layer, Müller cell fibers traversing the whole retinal tissue display immunolabeling for GFAP after light treatment (Fig. 1B). In control retinal tissues, Kir4.1 protein was concentrated around the vessels and at the limiting membranes (Fig. 1C). Except for a staining of Müller cell's microvilli which extend into the subretinal space in situ, the outer retina of control mice was largely devoid of Kir4.1 staining. Along with the degeneration of the photoreceptor cells, Kir4.1 immunoreactivity emerged in the outer retina between the remaining photoreceptor nuclei and around the Hoechst-stained large fusion bodies (Fig. 1C). Electrophysiological recordings in Müller cells isolated 44 days after light exposure revealed no significant alterations in the amplitude of potassium currents (114.8 ± 27.3% of control [100%]; P>0.05) and the resting membrane potential (-84.6 ± 2.2 mV) when compared to control cells (-84.0 ± 1.9 mV; P>0.05; Fig. 2A-C). However, an increase in the membrane capacitance of the cells (which is proportional to the cell membrane area) indicates a hypertrophy of the cells (Fig. 2D); cellular hypertrophy is a characteristic event when
Müller cells become reactive under various pathological conditions [3]. Similarly to Kir4.1, aquaporin-4 was stained very faintly in the outer retina of control mice. Aquaporin-4 was predominantly localized in the inner retina, within the ganglion cell and plexiform layers, and around the vessels (Fig. 1D). In the outer retina of controls, a staining for aquaporin-4 was observed in the outer plexiform layer and at the outer limiting membrane whereas the outer nuclear layer was largely devoid of aquaporin-4 immunoreactivity. After light exposure, the aquaporin-4 staining in the outer nuclear layer was increased. The co-staining of aquaporin-4 with the Müller cell marker glutamine synthetase indicates a glial expression of aquaporin-4 in the outer retina after light exposure (Fig. 1D). The data suggest that Müller glial cells respond to the local edema in the outer retina of bright white light-treated mice with a redistribution of Kir4.1 and aquaporin-4 proteins.

Exposure of the sensory retina to excessive light results in photoreceptor cell death and a damage to the pigment epithelium; both degenerative events contribute to the development of a local edema in the outer retina. Since Müller glial cells maintain the osmohomeostasis of the inner retina mainly via channel-mediated co-transport of potassium and water [2,3,9], we were interested to investigate whether these cells respond to the edema in the outer retina with alterations in the localization of potassium and water channel proteins, and in the potassium conductance. We found that excessive light re-activates Müller glial cells, as indicated by cellular hypertrophy and the increase in immunoreactive GFAP. Furthermore, we found that illumination with bright white light results in increases in the immunoreactivities for the glial water and potassium channels aquaporin-4 and Kir4.1 in the outer retina. Because the whole-cell potassium currents remained largely unaltered, the increase in the outer retinal immunostaining of Kir4.1 is supposed to be caused by a redistribution of the channel protein. Müller cells are suggested to dehydrate the inner retinal tissue by transcellular water fluxes driven by concomitant movements of potassium ions through Kir channels [2,3,9]. The simultaneous increase in the immunolabeling of aquaporin-4 and Kir4.1 channel proteins in the outer retina suggests a functional adaptation to the pathological situation. We propose that after light injury, Müller cells are active in a rapid and efficient clearance of excess water and ions extruded from the dying photoreceptor cells and extravasated through the damaged pigment epithelium, resulting in a clearance of the edema from the outer retinal tissue. It remains to be determined whether other subtypes of Kir channels previously implicated in glial potassium homeostasis [6] also alter their localization after light injury. Previously, we found no alteration in the localization of glial aquaporin-4 in the degenerating retina of rds mice [4]. Probably, the very slow, successive degeneration of photoreceptors in the retina of rds mice (which extends over several months and is not associated with a formation of large spherical bodies in the outer nuclear layer) fails to cause sufficient disturbances in the retinal osmohomeostasis to trigger an alteration in the expression of aquaporin-4 in Müller glial cells.

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References


Fig. 1. Treatment with bright white light alters the immunolocalization of GFAP, Kir4.1 and aquaporins in retinas of mice. The retinal slices were stained 3, 10, and 44 days after light exposure. (A) Aquaporin-1 immunoreactivity is predominantly expressed in the outer retina by the photoreceptor cells and segments. In the inner retina, aquaporin-1 staining was found in erythrocytes within the vessels. Progressive degeneration of photoreceptors is reflected by a decrease in aquaporin-1 immunostaining. (B) Immunostaining against the glial cell marker GFAP. In control tissues, astrocytes in the ganglion cell layer (GCL) display GFAP labeling. After light treatment, Müller cells express GFAP immunoreactivity. A similar alteration in GFAP immunolabeling was observed 3 and 10 days after light treatment. (C) Staining of Kir4.1. The arrows point to perivascular labeling, and the arrowheads indicate the inner and outer limiting membranes. The insets display the outer retina at higher magnification. (D) Staining against aquaporin-4. The insets display staining against aquaporin-4 (left) and co-staining of aquaporin-4 and the glial cell marker glutamine synthetase in the outer retina (right). The yellow merge signal indicates that Müller glial cells express aquaporin-4 around
photoreceptor cells. Cell nuclei were stained with Hoechst 33258 (blue). Note the presence of large spherical bodies in the outer nuclear layer (ONL) of degenerating retinas that were stained with the Hoechst dye. INL, inner nuclear layer; IPL, inner plexiform layer. PRS, photoreceptor segments. Bars, 20 µm.

Fig. 2. Treatment of mice with bright white light does not alter the potassium conductance of Müller glial cells but causes an increase in the cell membrane area. The data were obtained from Müller cells isolated three and 44 days after light treatment, as well as from Müller cells of untreated control retinas (co). (A) Representative traces of whole-cell currents in cells isolated from a control and a light-treated retina. The potassium currents were evoked by 20-mV incremental voltage steps between -180 and +20 mV from a holding potential of -80 mV. Outward currents are depicted upwardly, inward currents downwardly. (B) Mean amplitude of the inward potassium currents. (C) Resting membrane potential. (D) Cell membrane capacitance as indirect assessment of the membrane area of the cells. Each bar represents values obtained in 9 to 10 cells. Significant differences vs. untreated control: *P<0.05; ***P<0.001.