Available online at www.sciencedirect.com
SCIENCE DIRECT
Vision Research 46 (2006) 688–698
Vision Research
www.elsevier.com/locate/visres

Voltage-dependent ion channels in the mouse RPE: Comparison with Norrie disease mice ☆

Guido Wollmann [a,e,*], Steffen Lenzner [b], Wolfgang Berger [c], Rita Rosenthal [a], Mike O. Karl [d], Olaf Strauss [a,d]

[a] Institut fuer Klinische Physiologie, Charite—Universitaetsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany
[b] Max-Planck-Institut fuer Molekulare Genetik, Berlin, Germany
[c] Lehrstuhl Medizinische Molekulargenetik, Institut fuer Medizinische Genetik, Universitaet Zuerich, Zuerich, Switzerland
[d] Experimentelle Ophthalmologie, Klinik und Poliklinik fuer Augenheilkunde, Universitaetsklinikum Hamburg-Eppendorf, Hamburg, Germany
[e] Department of Neurosurgery, Yale University School of Medicine, New Haven, CT, USA

Received 27 February 2005; received in revised form 24 August 2005

Abstract

We studied electrophysiological properties of cultured retinal pigment epithelial (RPE) cells from mouse and a mouse model for Norrie disease. Wild-type RPE cells revealed the expression of ion channels known from other species: delayed-rectifier K^+ channels composed of Kv1.3 subunits, inward rectifier K^+ channels, $Ca_V1.3$ L-type Ca^{2+} channels and outwardly rectifying Cl^- channels. Expression pattern and the ion channel characteristics current density, blocker sensitivity, kinetics and voltage-dependence were compared in cells from wild-type and Norrie mice. Although no significant differences were observed, our study provides a base for future studies on ion channel function and dysfunction in transgenic mouse models.

Keywords: Retinal pigment epithelium; Ion channels; Norrie disease; Transgenic mouse model; Cell culture

1. Introduction

Placed between the outermost layer of the photoreceptors and the choroidal capillary layer, the retinal pigment epithelium (RPE) acts as a key element of the blood retinal barrier (Bok, 1993). It is involved in the regulation of the nutrient flow and substrate exchange, phagocytosis and recycling of shed photoreceptor outer segment disks and retinoid metabolism (Finnemann, 2003; Thompson & Gal, 2003; Young & Bok, 1969), secretion of growth factors (Tanihara, Inatani, & Honda, 1997), and homeostasis of the ion composition in the subretinal space (Steinberg, 1985). Altered RPE–photoreceptor interaction may cause or intensify a variety of retinal diseases, as has been shown in age-related macular degeneration (AMD) (Lutty, Grunwald, Majji, Uyama, & Yoneya, 1999) or retinitis pigmentosa (Strauss, 2005).

In numerous studies, the expression and functional role of voltage-dependent ion channels in the RPE of many species including man have been described. These ion channels are comprised of outwardly rectifying K^+ channels such as delayed rectifier (Hughes, Takahira, & Segawa, 1995; Strauss et al., 2002), M-type currents (Tanihara et al., 1997) and Ca^{2+}-dependent K^+ channel (Tao & Kelly, 1995), inward rectifier K^+ channel (Hughes & Takahira, 1996), L-type Ca^{2+} channels (Strauss, Mergler, & Wiederholt, 1997; Ueda & Steinberg, 1993) and several types of outwardly rectifying Cl^- channels (Strauss, Steinhausen, Mergler, Stumpff, & Wiederholt, 1999; Wills et al., 2000). The delayed rectifier K^+ channels appeared to be mainly composed of Kv1.3 subunits (Pinto & Klumpp, 1998),

☆ Grant information: Supported by Deutsche Forschungsgemeinschaft Grants STR480/8-2 and STR480/9-1.
* Corresponding author. Tel.: +1 203 785 3143; fax: +1 203 737 2159.
E-mail address: guido.wollmann@yale.edu (G. Wollmann).

0042-6989/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.visres.2005.08.030

the inward rectifier is mainly composed of Kir 7.1 subunits (Shimura et al., 2001) and the L-type Ca^{2+} channel is of the $Ca_V1.3$ subtype which is determined by the expression of α_{1D} subunits (Strauss et al., 2000).

Recently, a knock-out mouse model was introduced which resembles the phenotype of Norrie disease (ND) in human (Berger et al., 1996). This neurodegenerative condition is a rare X-linked recessive disorder characterized by congenital blindness, mental retardation and progressive sensorineural hearing loss (Warburg, 1966). The mechanisms causing the retinal degeneration remain unclear and a possible involvement of altered RPE-function has not been elucidated so far. Recent morphological, physiological and molecular studies revealed pathological changes in the inner retina and vitreous (Berger, 1998). However, alterations like a mild ERG phenotype, pigment mottling and progressive degeneration of photoreceptor cells may also point to the outer retinal layers (Berger, 1998; Lenzner et al., 2002; Ruether et al., 1997). Therefore, involvement of the RPE in the pathophysiology of this disease is possible too.

In the present work, we addressed two points. First, we investigated the pattern of ion channels in mouse RPE using the patch-clamp technique, Western blot and immunocytochemistry. For the first time, we could present data of mouse RPE cells, that were successfully maintained in primary culture. The second aspect was the electrophysiological comparison of cultured RPE cells from ND-knock-out mice and wild-type mice to shed light into a potential involvement of the RPE in Norrie disease.

2. Materials and methods

2.1. Animals

Norrie mice were generated by homologous recombination in embryonic stem cells of mouse strain 129 (Berger et al., 1996). The resulting mouse line was bred within the C57BL/6 background for at least five generations. The knockout line is lacking the coding part of exon two of the mouse *Ndph* (Norrie disease pseudoglioma homolog) gene, including the ATG translation start codon. Status of the Norrie gene and mouse gender were examined by PCR using DNA derived from tail tissue of each mouse sacrificed; status was blinded for the duration of the patch-clamp studies.

Animals were treated in accordance to the ARVO statement for the use of animals in ophthalmic and vision research and the regulation for animal experiments of the federal government of Germany.

2.2. Cell culture

Eyes from 12–14 day-old mice were enucleated and processed following an adapted protocol from Edwards for establishing primary cultures from retinal pigment epithelium cells (Edwards, 1977). After an overnight incubation in Puck's saline F, eyes were treated in trypsin solution (0.1% in Puck's saline without Ca^{2+} and Mg^{2+}) for 30 min at 37 °C. The bulbi were incised along the ora serrata and the anterior part and the vitreous were removed. The retinal layer was carefully stripped off from the flattened posterior compartment and a single layer RPE sheet was isolated from the underlying Bruch membrane and collected in Ham's F10 culture medium supplemented with 20% fetal calf serum, 100 μg/ml kanamycin and 50 μg/ml gentamycin. Cells were dissociated by repeated pipetting. The cell suspension was then plated onto 12 mm glass cover slips inside a 60 mm cell culture dish. Attached cells started to spread out after 24–48 h. Cultures were maintained at 37 °C and 5% CO_2 in air; medium was changed twice a week. Cell culture media and supplements were purchased from Biochrom (Berlin, Germany).

2.3. Patch-clamp recordings

Patch-clamp recordings were performed at room temperature. Cover slips with semi-confluent RPE cultures aging between 4 and 12 days in culture were placed into a perfusion chamber mounted onto the stage of an inverted microscope. Only cells with a bright and healthy appearance were approached. For recording of potassium channels, the cells were superfused with a bath solution containing (in mM): 130 NaCl, 3 KCl, 0.3 $CaCl_2$, 0.6 $MgCl_2$, 14 $NaHCO_3$, 1 $NaHPO_4$, 33 *N*-2-hydroxyethylpiperazine-*N*′-2ethanesulfonic acid (Hepes), and 5.5 glucose, adjusted to pH 7.2 with Tris. Studies of Ca^{2+} and Cl^- conductances were performed in a K^+-free bath solution consisting of (in mM): 130 NaCl, 3 TEACl, 0.2 $CaCl_2$, 10 $BaCl_2$, 0.6 $MgCl_2$, 14 $NaHCO_3$, 1 $NaHPO_3$, 33 Hepes, 5.5 glucose, adjusted to pH 7.2 with Tris. $BaCl_2$ served as charge carrier to measure currents through L-type Ca^{2+}-channels. Patch-clamp electrodes were pulled from borosilicate glass tubes using a universal puller (DMZ; Zeitz, Augsburg, Germany) and showed a resistance of 3–5 MΩ. The pipette solution used for K^+-channel experiments contained (in mM): 100 KCl, 10 NaCl, 0.5 $CaCl_2$, 2 $MgSO_4$, 10 Hepes, adjusted to pH 7.2 with Tris. Pipettes used for experiments to measure Ca^{2+}- and Cl^--currents were filled with (in mM): 100 CsCl, 10 NaCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5.5 EGTA–Tris, and 10 Hepes. Currents through K^+-channels were recorded in the conventional whole-cell configuration and Ca^{2+}- and Cl^--conductances were measured using the perforated patch approach (150 μg/ml nystatin in pipette solution). To avoid hyperosmotic swelling of the cells, the pipette solution was approximately 60 mOsm hyposmotic to the bath. Osmolarity was measured using a vapor pressure osmometer (model 5100 B; Wescor, Logan, UK). No changes in cell size were observed during the whole-cell configuration with these solutions. Membrane currents were recorded using an EPC-7 patch-clamp amplifier (HEKA; Lamprecht, Germany) and low-pass-filtered at 3 kHz. An AT compatible computer and TIDA software (HEKA; Lamprecht, Germany) were used for electrical stimulation as well as for data storage and analysis. The membrane capacitance and access resistance were compensated after the whole-cell configuration was established. The access resistance was compensated