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Relationship between rat retinal degeneration and potassium channel KCNQ5 expression

Elena Caminos [a, *], Cecilia F. Vaquero [b], Juan R. Martinez-Galan [a]

[a] School of Medicine and Institute for Research in Neurological Disabilities (IDINE), University of Castilla-La Mancha, Albacete, Spain
[b] School of Medicine and Regional Center for Biomedical Research (CRIB), University of Castilla-La Mancha, Albacete, Spain

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ABSTRACT

KCNQ5/Kv7.5 is a low-threshold non-inactivating voltage-gated potassium channel preferentially targeted to excitatory endings in brain neurons. The M-type current is mediated by KCNQ5 channel subunits in monkey retinal pigment epithelium cells and in brain neurons. This study was undertaken to analyze KCNQ5 expression and the interaction signals of KCNQ5 with other proteins in normal rat retina and during photoreceptor degeneration. The KCNQ5 expression pattern was studied by immunocytochemistry and Western blot in normal rat retinas (Sprague–Dawley, SD) and P23H-1 rats as a retinitis pigmentosa model. The physical interactions of KCNQ5 with calmodulin (CaM), vesicular glutamate transporter 1 (VGluT1) and glial fibrillary acidic protein (GFAP) were analyzed by *in situ* proximity ligation assays and were supported by calcium recording. KCNQ5 expression was found in the plexiform layers, ganglion cell layer and basal membrane of the retinal pigment epithelium. The physical interactions among KCNQ5 and CaM, VGluT1 and GFAP changed with age and during retinal degeneration. The maximal level of KCNQ5/CaM interaction was found when photoreceptors had almost completely disappeared; the KCNQ5/VGluT1 interaction signal decreased and the KCNQ5/GFAP interaction increased in the inner retina, while degeneration progressed. The basal calcium levels in the astrocytes and neurons of P23H-1 were higher than in the control SD retinas. This study demonstrates that KCNQ5 is present in the rat retina where its activity may be moderated by CaM. Retinal degeneration progression in P23H-1 rats can be followed by an interaction between KCNQ5 with CaM in an *in situ* system. The relationship between KCNQ5 and VGluT1 or GFAP needs to be more cautiously interpreted.

1. Introduction

The initial interest in studying the potassium channel KCNQ5 (or Kv7.5) in the retina was based on the electrophysiological data obtained from rods and retinal pigment epithelium (RPE) cells where rectifying K^+ currents were identified (Beech and Barnes, 1989; Pattnaik and Hughes, 2012). These K^+ currents were inhibited by XE991 (a KCNQ channel blocker) or activated with retigabine (a KCNQ channels opener) in RPE cells (Pattnaik and Hughes, 2012), as resembling those M-type currents in excitable cells in which KCNQ5 is implicated (Lerche et al., 2000; Schroeder et al., 2000). It has not yet been demonstrated that KCNQ5 contributes to voltage-gated K^+ channels in inner segments of photoreceptors and other retinal cells. However, the results obtained in the present study support this possibility.

KCNQ/Kv7 is a family of five potassium channels involved in the control of cellular excitability in the heart, inner ear and central neurons. Mutations in KCNQ1-4 genes, but not in KCNQ5, underlie several human pathologies (Biervert et al., 1998; Kubisch et al., 1999; Wang et al., 1996). KCNQ5 has three splice variants of different sizes. KCNQ5-v1 encodes a protein that is 932 amino acids long; KCNQ5-v2 encodes the truncated form which affects nine amino acids in the C-terminus domain, KCNQ5-v3 encodes a protein with 19 extra amino acids (Schroeder et al., 2000; Yeung et al., 2008). A study conducted in monkey retina has reported that RPE cells express KCNQ5-v2 and that the neural retina expresses KCNQ5-v1 and -v2 (Zhang et al., 2011). KCNQ5-v2 expression has also been found in bovine RPE, where it is implicated in the K^+ concentration control in subretinal fluid, which is important to maintain the functions of photoreceptors, Müller cells and also RPE

* Corresponding author. Department of Medical Sciences, School of Medicine, University of Castilla-La Mancha, C/ Almansa 14, 02006 Albacete, Spain.
E-mail addresses: elena.caminos@uclm.es (E. Caminos), cecilia.fernandez@uclm.es (C.F. Vaquero), juanramon.martinez@uclm.es (J.R. Martinez-Galan).

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cells (Zhang and Hughes, 2013). To date, no functions in relation to the presence of KCNQ5 in neural retina have been assigned or suggested.

The biophysical and pharmacological features of KCNQ5 can be altered by associated proteins, such as Src tyrosine kinase, KCNE1 or KCNE3, which alter the potassium current (Gamper et al., 2005; Roura-Ferrer et al., 2009), or calmodulin, which modulates potassium channel activity by interacting with the C-terminal domain of KCNQ5 (Gamper et al., 2005; Yus-Najera et al., 2002). The distribution of KCNQ5 in excitatory endings of central auditory neurons can also be altered if the inputs to these neurons are not intact (Caminos et al., 2007; Garcia-Pino et al., 2010). Some studies have also suggested that glial cells express KCNQ5 and that the channel is involved in glial cell maturation (Wang et al., 2011; Yus-Nájera et al., 2003).

The first goal of this study was to check whether KCNQ5 is expressed in a normal rat retina and in rats with retinal degeneration. The P23H-line 1 (P23H-1) transgenic rat undergoes gradual, fast photoreceptor loss, which is characteristic of autosomal dominant retinitis pigmentosa, with progressive changes occurring in the connectivity patterns of photoreceptor pathways (Cuenca et al., 2004; Machida et al., 2000). In this study, we also analyzed the co-localization and subsequent possible physical interaction of KCNQ5 with a protein involved in channel activity in the brain (calmodulin, CaM) and the proteins affected by retinal degeneration progression (the vesicular transporter of glutamate 1, VGluT1, and glial fibrillary acidic protein, GFAP).

2. Materials and methods

2.1. Animals

Transgenic P23H-1 homozygous albino rats were kindly provided by Dr. Matthew LaVail (UCSF School of Medicine, Beckman Vision Center, San Francisco, CA, USA), and were bred in a colony at the University of Castilla-La Mancha (UCLM, Albacete, Spain). Sprague–Dawley (SD, Charles River Laboratories, Barcelona, Spain) rats were used as the wild-type controls. All the animals were housed and handled under the authorization and supervision of the animal facility staff at the UCLM. Protocols were approved by the UCLM Ethics Committee for Experimental Animal Welfare. This study was conducted in accordance with European and Spanish laws (Directive 2010/63/UE and RD 53/2013).

2.2. Western blot

Anesthesia was induced with 4% isoflurane (1 L/min O_2 flow rate) into an appropriate chamber prior to decapitation. Eyes were removed, and retinas were isolated and frozen in liquid nitrogen and stored at −80 °C. The eyes from at least three animals per age (P20, P90, P110 and 200) of each rat strain were used. Retinas were homogenized in 2 ml of ice-cold lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 1 mM NaF, 1% Triton, 20 mM PMSF, and 20 μg/ml of each aprotinin and leupeptin). Homogenates were centrifuged at 12,000 *g* for 10 min at 4 °C and supernatants were stored at −80 °C. Protein concentration was determined with the BCA Protein Assay kit (Thermo Fisher Scientific, Rockford, IL, USA). Eighteen μg of whole protein extracts were electrophoresed on 10% SDS polyacrylamide gels using the mini-PROTEAN III system (Bio-Rad, Hercules, CA, USA) for 90 min at 120 V. Gels were transferred onto nitrocellulose membranes (GE Healthcare Bio-Sciences, Uppsala, Sweden) for 2 h at 120 V using a semidry blotter (Bio-Rad). Immunodetection was performed by blocking nonspecific binding in the blots with Tris-buffered saline-Tween-20 (TBST: 50 mM Tris pH 7.5, 200 mM NaCl, 0.1% Tween 20 and 5% nonfat dry milk) for 1 h, at room temperature (RT). Then membranes were incubated with anti-KCNQ5 (Table 1) in TBST, overnight at 4 °C, and subsequently with the secondary antibody (Table 1) for 1 h at RT. Next membranes were developed with a chemiluminescence assay (SuperSignal West Dura Extended Duration Substrate, Pierce, Rockford, IL, USA) for 5 min and were scanned in a computer equipped with the LAS-mini 3000 system (Fujifilm, Tokyo, Japan). Finally, membranes were incubated in stripping buffer (Pierce) at 37 °C for 30 min and were exposed to anti-GAPDH (Table 1). The controls included: (1) blots with anti-KCNQ5 partially preabsorbed with the antigenic KCNQ5 protein (1:20; Abnova); and (2) extracts of cerebellum and cochlear nucleus, as published previously (Garcia-Pino et al., 2010).

2.3. Immunofluorescence

SD ($n = 17$) and P23H-1 ($n = 15$) rats, aged 20, 60, 90, 160 and 200 postnatal days (P), were used. At least three animals per age and strain were anesthetized with an intraperitoneal injection of a mixture of ketamine (100 mg/kg, Parke-Davis, Alcobendas, Spain) and 2% Xylazine (10 mg/kg, Dibapa, Barcelona, Spain), and were transcardially perfused with 0.9% saline and 2% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.3, as published previously (Garcia-Pino et al., 2010). Eyes were dissected, postfixed for 4 h in the same fixative, and washed and transferred into PB containing 30% sucrose. Fifty six eyes were embedded in Tissue Tek (Leica,

Table 1
Primary and secondary antibodies used for immunocytochemistry (IR) and Western blot (WB).

Antigen	Immunogen	Host species/ mono-polyclonal	Manufacturer/ Catalog#/Clone	Dilution
Primary antibodies				
KCNQ5 (or Kv7.5)	Fusion proteins from transformed *E. coli* strains	Rabbit/ polyclonal	Millipore (Temecula, CA)/# AB5599	IR: 1:1000 WB: 1:2000
Calmodulin	Synthetic peptide of bovine calmodulin	Mouse/ monoclonal	Millipore (Temecula, CA)/# 05-173	IR: 1:500
Vesicular glutamate transporter (VGluT1)	Fusion protein of rat VGluT1	Mouse/ monoclonal	NeuroMab (Davis, CA)/# 75-066/Clone N28/9	IR: 1:500
GFAP	Glial Fibrillary Acidic Protein from pig spinal cord	Mouse/ monoclonal	Sigma (St. Louis, MO, USA)/#G3893/Clone G-A-5	IR: 1:500
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Purified rabbit muscle GAPDH	Mouse/ monoclonal	Applied Biosystem (Foster City, CA)/# AM4300/Clone 6C5	WB: 1:4000
Secondary antibodies				
Cy2-conjugated goat anti-rabbit ($F(ab')_2$/Fab portion)			Jackson ImmunoResearch (Baltimore Pike, PA)/ # 111-226-047	IR: 1:500
Cy5-conjugated goat anti-Mouse IgG (H + L)			Jackson ImmunoResearch (Baltimore Pike, PA)/ # 115-175-146	IR: 1:500
Peroxidase conjugated goat anti-rabbit IgG (H + L)			Vector Laboratories (Burlingame, CA)/# PI-1000	WB: 1:1000
Peroxidase conjugated horse anti-mouse IgG (H + L)			Vector Laboratories (Burlingame, CA)/# PI-2000	WB: 1:2000