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Plasticity of TRPM1 expression and localization in the wild type and degenerating mouse retina

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ABSTRACT

The light response in retinal ON bipolar cells is associated with disinhibition of current flow through cation channels recently identified as type 1 members of the melastatin transient receptor potential (TRPM) family. We determined the developmental expression of *Trpm1* in the wild type C57BL/6, DBA/2J, DBA2J-*Gpnmb* mouse retinas and in *Pde6b*^{rd1} retinas characterized by degeneration of rod photoreceptors. *Trpm1* mRNA in wild type retinas was low at birth but exhibited progressive increases in abundance up to early adulthood at postnatal day 21 (P21). Retinal *Trpm1* mRNA content did not decrease following loss of photoreceptors. At P21, TRPM1-immunopositive perikarya migrated into the outer nuclear layer. The TRPM1 protein was trafficked to discrete postsynaptic puncta in wild type retinas whereas in adult *Pde6b*^{rd1} mouse retinas, TRPM1 translocated to bipolar perikarya and bar-like structures in the distal inner nuclear layer. These findings show that expression and localization of the *TRPM1* in the mouse retina is plastic, modulated by use-dependence and availability of sustained excitatory input.

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1. Introduction

Glutamate, released from vertebrate photoreceptors, activates retinal ON bipolar neurons through a sign inverting mechanism mediated by the GRM6–Goα/Gβ5-nyctalopin-RGS7/11 transduction pathway (Chen et al., 2010; Dhingra et al., 2002; Nomura et al., 1994; Rao, Dallman, Henderson, & Chen, 2007; Zhang et al., 2010). While all the steps in the ON bipolar transduction cascade remain to be determined, the main cation channel downstream from the heteromeric G protein has been identified as TRPM1, a member of the melastatin TRP channel family (Koike et al., 2010; Morgans et al., 2009). Mice, equines and humans lacking functional TRPM1 develop congenital stationary night blindness (CSNB2), a multigene disease associated with loss of the ERG b-wave (Bellone et al., 2008; Nakamura et al., 2010; Shen et al., 2009; van Genderen et al., 2009). Genetic elimination of *Trpm1* compromised ON bipolar transduction in mice (Morgans et al., 2009) whereas heterologous expression of mGluR6, Goα and TRPM1 reconstituted a nonselective cation channel that was negatively regulated by glutamate (Koike et al., 2010). Nonetheless, the precise mechanism underlying TRPM1 transduction remains to be determined because a proportion of these channels might be localized to intracellular, not plasmalemma, compartments (Oancea et al., 2009; Patel & Docampo, 2009). As a first step towards an integrated view of TRPM1 function in the mouse retina, we studied the plasticity of *Trpm1* expression during development and degeneration.

A previous microarray study suggested that transcription of the *Trpm1* gene in the mouse retina increases transiently at postnatal day 6 (P6) and returns to the baseline at P10 (Kim et al., 2008). Given that the *Trpm1* transcript levels peak early in postnatal development when synaptic connections and transmission are not yet fully functional (Kim et al., 2008) yet TRPM1 plays an indispensable function in adult vision (Morgans et al., 2009), we decided to re-examine this process across the entire developmental sequence of the wild type mouse retina. To assess if the gene is affected by the loss of bipolar inputs and outputs, we also studied *Trpm1* expression in *Pde6b*^{rd1} (*rd1*) and DBA/2J retinas in which rod photoreceptors and retinal ganglion cells underwent degeneration. In the *rd1* retina the majority of rod photoreceptors die in the first postnatal week, leading to severe changes in cellular architecture and remodeled neural circuitry in the retina (Carter-Dawson, LaVail, & Sidman, 1978; Farber, Flannery, & Bowes-Rickman, 1994; Jones et al., 2003). The deafferentiation is associated with remodeling of postsynaptic sites on bipolar cells and an increased glutamatergic drive in the inner retina (Marc et al., 2007; Margolis, Newkirk, Euler, & Detwiler, 1998; Stasheff, 2008; Strettoi &

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Pignatelli, 2000). It is believed that the canonical mGluR6 pathway that defines ON bipolar cells disappears following deafferentiation. Surprisingly, however, our results demonstrate that retinal *Trpm1* transcript and protein levels are maintained following major photoreceptor loss. Such maintained expression of *Trpm1* and *Grm6* genes may preserve the ability of the ON bipolar transduction mechanisms in the deafferented inner retina to respond, under circumstances yet to be determined, to glutamatergic signals.

2. Materials and methods

2.1. Animals

Breeding pair founders for C57BL6 wild type, DBA/2J, D2.B6-*Tyrp1B6GpnmbB6*/Sj (hereafter referred to as DBA-*Gpnmb*) and *Pde6b^{rd1}* mice were obtained from Jackson Laboratories (Bar Harbor, ME). The wild type and *Pde6b^{rd1}* animals were on the same background, as were the DBA/2J and DBA-*Gpnmb* animals.

2.2. In situ hybridization

In situ hybridization and probe synthesis were performed as described (Punzo & Cepko, 2007; IOVS (48) 849–857). Sp6 RNA polymerase was used to generate the probes. The probes for *Trpm1* were generated by sub-cloning part of the coding sequence into pGEMT-Easy (Promega). The forward primer was: CAGGGTCAGA AAGCATGGAT; reverse: CCCAGCCTTGTGTTGATCTT. The identity of the gene was verified by sequence analysis. For paraffin sections, retinas were fixed for 30 min in 4% PF/PBS at RT, washed with PBS and dehydrated to 100% ethanol using a ladder of increased EtOH concentrations before embedding in 50/50 xylene/paraffin (60 ; 15 min) and 100% paraffin (4 × 30 min at 60 °C).

2.3. Semi-quantitative real time PCR

Total RNA from retina was extracted with Trizol and total RNA was converted to cDNA using the SuperScript III First-Strand Synthesis kit from Invitrogen. Real time PCR was performed on a thermocycler (GeneAmp 5700; ABI, Foster City, CA) using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) reagents according to the manufacturer's instructions. The probes were: *Trpm1* (exons 4–5): Forward: AAAGGAGGATGAGCAGCAGTCGTC; Reverse: ATTCTCCAGAGCG CTCACCATCCT; *Grm6* (exons 6–7): Forward: CAGCCATGGAGCCTACTGATGG; Reverse: CACTGCTGGCACT TCCATTGG; *CACNA1F*: Forward CTGGGCCGAGTG ATGATGATGG; Reverse: TCTGATTGTCCTGCCCTTGAGTTC; *Vglut2*: Forward GACATAGT GGAACAGGAAAGATGG; Reverse TTTCACAAAACACTGCAAGATA AGCT; *Rho*: Forward GCCTCAGTCTGCATCCCTCCTCTA; Reverse AAG CGTCCC AGTTTCCATCCATT. Amplification of PCR products was measured by fluorescence associated with binding of double-stranded cDNA to SYBR Green in the reaction mixture. After an initial denaturation step of 50 °C for 2 min and 95° for 10 min, PCR reaction was repeated for 40 cycles at 95 for 15 s, 1 cycle at 58 for 30 s, and 1 cycle at 72 °C for 30 s. After amplification, the ratio of gene-of-interest mRNA to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) reference gene was calculated for each sample. A random sample at the earliest age of interest was assigned a value of 1 and other values calculated relative to the sample. Every experiment consisted of samples from at least three animals, each gene was studied in 3–5 separate experiments. The experiments in Fig. 3A were conducted on separate PCR plates, hence DBA/2J values for *Vglut2* are compared to normalized C57BL/6 values for each age. Statistical significance was determined with the two-tailed nonparametric Mann–Whitney test.

2.4. Immunohistochemistry

Enucleated eyecups with the retinas were immersion-fixed for 1 h in 4% (w/v) paraformaldehyde in phosphate buffer (PB; 0.1 M; pH = 7.4). The retinas were rinsed two times in PB, cryoprotected in 15% and 30% sucrose overnight at 4 °C, mounted in OCT and cryostat sectioned at 16 μm. Retinal sections were washed in PB for 15 min, then permeabilized and blocked in a solution containing 0.5% Triton X-100 and 10% goat serum. The TRPM1 antibody, used at 1:100, has been characterized previously (Koike et al., 2010). Antibodies raised against the synaptic vesicle marker SV2 (K. Buckley; Developmental Studies Hybridoma Bank, Univ. of Iowa) and protein kinase C α subunit (PKCα, clone MC5, Santa Cruz Biotechnology) were used at 1:100. The cone marker peanut agglutinin lectin conjugated to Alexa Fluor 594 nm (PNA; Invitrogen) was used at 1:10 dilution. We utilized the following secondary antibodies: AlexaTM 488 and AlexaTM 594 nm goat anti-mouse or goat anti-rabbit IgG (H + L) conjugates (Invitrogen), diluted at 1:1000. After incubation, sections were washed in PB and covered with Vectashield (Vector, Burlingame, CA). The immunolabeled sections were examined by confocal microscopy (Zeiss LSM 510). In double-labeled experiments, the images were acquired separately from each laser channel (488 Ar or 546 HeNe lines), then recombined. Adjustments of contrast and intensity were made in Photoshop (Adobe, San Jose, CA) and were uniform across the entire image.

3. Results

The majority of bipolar neurons in the mouse retina develop in the first week after birth (Young, 1985) whereas their synaptic contacts mature between the eye opening and the third postnatal week (P12–P21; Fisher, 1979; Olney, 1968). Using semi-quantitative RT-PCR, *Trpm1* mRNA content was analyzed in the postnatal mouse retina to determine whether expression of the *Trpm1* gene matches bipolar perikaryal and/or synaptic development. As illustrated in Fig. 1, low levels of *Trpm1* mRNA in wild type control retinas were detected in the first week after birth. *Trpm1* mRNA levels increased around the time of eye opening (P12–P14; black bars in Fig. 1), reaching adult levels at P21. In situ hybridization analysis showed strong signal with the antisense probe in the distal INL, whereas little expression was detected in other retinal layers (Fig. 1B), indicating that the *Trpm1* signal is predominantly confined to bipolar neurons. Increased expression of the *Trpm1* gene in the developing mouse retina is consistent with gradual development of bipolar synapses (Fisher, 1979) and excitatory neurotransmission (Tian & Copenhagen, 2001) in the first week after the eye opening.

Retinal degenerations in mouse models of retinitis pigmentosa are characterized by dendritic outgrowth of deafferented bipolar and horizontal neurons (Jones et al., 2003; Strettoi, Pignatelli, Rossi, Porciatti, & Falsini, 2003) and by large-scale rhythmic bursting in postsynaptic neurons triggered by the loss of bipolar input (Stasheff, 2008; Margolis et al., 1998). To determine whether the *Trpm1* message is affected by remodeling processes in the outer retina, we analyzed *Trpm1* mRNA content in the *Pde6b^{rd1}* retinitis pigmentosa mouse model in which the large majority of rods degenerate by P21 (Carter-Dawson et al., 1978; Lolley, 1974). The ON bipolar neurons show extensive remodeling response in *Pde6b^{rd1}* retinas in which they extend dendrites into the ONL in search of glutamatergic inputs (Strettoi & Pignatelli, 2000). While adult *Pde6b^{rd1}* retinas were associated with near complete loss of rhodopsin and a reduction in the content of *Cav1.4* L-type channel transcripts (Fig. 1D and E), prominent *Trpm1* mRNA signatures were observed in surviving *rd1* bipolar neurons (Fig. 1C) and retinas (Fig. 1A). Expression of the

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