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Dark-Rearing the *rd10* Mouse: Implications for Therapy

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Keywords

Dark-rearing; Phosphodiesterase 6b; *rd10* Mouse model; Rod ON-bipolar cells; Electroretinogram

18.1 Introduction

Gene-based, pharmacological, and prosthetic therapies have been investigated for the prevention or reversal of photoreceptor cell death in retinal degeneration (RD). Retinitis pigmentosa (RP) and allied forms of RD can be triggered by a wide range of deleterious mutations, over 170 identified thus far: in the retina it seems “anything that can go wrong will go wrong.” This calls for several therapeutic approaches, which in the absence of alternatives have to be tested in mouse models. The efficiency of the test depends on the model; it should neither degenerate too fast nor too slow. In the extensively studied *rd1* mouse (Bowes et al. 1990; Punzo and Cepko 2007), the effects of the phosphodiesterase 6b (*PDE6β*) mutation are so severe, arising between PN5 and PN14, that several layers of photoreceptor cells are lost before the retinal circuitry has reached full maturation. By contrast, the relatively recently characterized *rd10* mouse shows the first signs of degeneration at PN18 (Chang et al. 2002). For this reason, the *rd10* model is of great value for early-stage therapeutic testing, balancing a significant retinal pathology with a slower rate of progression and thus the opportunity to achieve rescue. Such a rescue strategy should depend on the nature of the mutation, whether it precipitates a null, leaky, gain-of-function, or dominant-negative phenotype. In the case of the *rd10* mouse, the *PDE6β* point mutation changes codon 560 of exon 13 from an arginine to a cysteine.

Although the pathophysiological mechanism of this *rd10* mutation has yet to be elucidated, the gradual degeneration of the *rd10* phenotype is characteristic of a leaky, haplo-insufficient state, as further implied by complementarity mating with wild-type (WT) and *rd1* mice (Chang et al. 2007). This suggests that a *PDE6β* gene replacement approach should achieve rescue. The therapeutic window available may be extended by dark-rearing the mouse from birth, a unique feature of the *rd10* retina that was originally reported by Chang and colleagues (2007) (Pang et al. 2008). This protective effect of dark-rearing has yet to be explained and may result from at least three possible mechanisms, (1) a gain-of-function in

the *rd10* *PDE6 β* molecule may cause light-evoked activity to be toxic to the cell, (2) altered synaptic development, as may occur by dark-rearing (Vistamehr and Tian 2004), may mask the retinal phenotype, (3) secondary cues for cell death may be accelerated by light and delayed by dark-rearing. In this preliminary study, the ERG, histology, and transcript levels of crucial retinal genes are compared between cyclic-light-reared and dark-reared mice, termed LR-*rd10* and DR-*rd10*, respectively. The data show that the ONL of the DR-*rd10* mouse is protected from cell death with some ONL function preserved until at least PNM2.5. Furthermore, the downstream circuitry of the rod ON-bipolar cells (RBCs) is maintained in the DR-*rd10* mice. The protective effect of light restriction in the *rd10* mouse may provide insight into disease management and treatment strategies for patients with retinal degeneration caused by recessive photoreceptor mutations.

18.2 Methods

18.2.1 Mice

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania and complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. C57Bl/6J and *PDE6 β ^{rd10}/J* mice were obtained from Jackson laboratories (ME).

18.2.2 Electroretinography

ERG recordings followed procedures described previously (Lyubarsky et al. 2002). Wild-type C57Bl/6J mice were dark-adapted overnight; *rd10* mice have been raised in dark from birth. Animals were anesthetized with a cocktail containing (in mg/g body weight): 25 ketamine, 10 xylazine, and 1000 urethane. The pupil was dilated with 1% tropicamide saline solution (Mydracil; Alconox, New York, NY), and the mouse was placed on a custom-made thermo-stabilized stage maintained at 37°C. Two miniature cups with embedded platinum wires made of UV-transparent plastic serving as recording electrodes were placed in electrical contact with the corneas.

A platinum wire loop placed in the mouth served as a reference and a ground electrode. Full-field ERG were recorded with Espion Electrophysiology System (Diagnosys LLC, Lowell, MA) modified by the manufacturer for experiments with mice by substituting LEDs with emission maximum at 365 nm for standard blue cones. Intensities of green and white stimuli were defined in scotopic units according to the manufacturer's instruction, and they were converted into the units of photon flux and number of photoisomerizations/photoreceptor as described by Lyubarsky et al. (2004).

18.2.3 Immunolabeling and Imaging

Immunolabeling of retinal cryosections was carried out according to previously described protocols (Sherry et al. 2006; Phillips et al. 2010). Antibodies used were rabbit PKC α (1/1000, Sigma, St. Louis, MO), rabbit VAMP-2 (1/500, ab3347 Abcam, MA), rabbit synapsin-1 (1/500, ab8), all raised in rabbit and detected using alexafluor-labeled secondary anti-rabbit antibodies (1/500, Molecular Probes, OR). Specificity of labeling methods was confirmed by omitting primary antibody. Confocal microscopy was performed using a Leica

confocal microscope. Images were captured using a 40× oil immersion objective and stacked images processed using Image J.

18.2.4 Quantitative Real-Time PCR

RNA samples were harvested from whole eyes using RNeasy kit (Qiagen) and used for single-strand cDNA synthesis from oligoDT primers. Primers amplifying a 150–200 bp amplicon of genes in enriched sets were designed using the Whitehead Institute Primer3 program. Thermal cycler conditions for the RTPCR system (Applied Biosystems 7500) were 50°C for 2 min, 95°C for 10 min, 40 cycles of 15 s at 95°C followed by 1 min at 60°C. PCRs were performed in triplicate for each sample and relative quantitation was calculated using the Pfaffl formula with 18S rRNA as reference.

18.3 Results

18.3.1 Preservation of Function Until PNM3 in the DR-*rd10* Mouse

The retinal function of DR-*rd10* was compared to that of LR-*rd10* mice using flash ERG at a range of ages from postnatal day (P) 21 to postnatal month (PNM) 3. The saturating a-wave response from dark-adapted mice is reduced compared to WT for both DR-*rd10*s and LR-*rd10*s, from P21; however, the DR response amplitude is twice that of the LR response and is maintained until at least 7 weeks long after the a-wave of LR-*rd10*s is lost. Even more striking is a dark-adapted b-wave response close to that of the WT, suggesting that not only is downstream synaptic signaling intact, but may even be undergoing a compensatory effect, with a b/a ratio increased compared to WT.

18.3.2 Immunohistochemical Analysis of the Effect of DR on *rd10* Retinal Remodeling

There is considerable evidence for anatomical reshaping of inner retinal neurons during retinal degeneration. Early disorganization and alteration in size of rod bipolar cell (RBC) terminals has been reported in *rd10* mice at PN30 (Puthussery et al. 2009). In both DR-*rd10* as well as LR-*rd10* mice, we observe remodeling of the RBC dendrites. At this advanced stage, cone outer segments are evident only in DR-*rd10* mice (Fig. 18.2a). It was of interest to examine the extent to which synaptic connections of the inner retina may be influencing the rate of degeneration in the DR-*rd10* mouse. Antibody against VAMP-2, the principal SNARE protein of retinal synapses, stains both ribbon and conventional synapses in the outer and inner plexiform layers (OPL and IPL), (Phillips et al. 2010). While VAMP-2 staining is completely diminished in the OPL of the *rd10* mouse reared under cyclic light, staining is maintained in the dark-reared *rd10* (Fig. 18.2b). Antibody against synapsin-1 labels the conventional synapses of amacrine cells in the IPL, previously synapsin-1 labeling had been observed ectopically in puncta of the INL and GCL of *rd10* retinas (Phillips et al. 2010). Spread of this synaptic marker into the OPL is observed in LR-*rd10* mice. By contrast, a more intense and confined labeling of conventional synapses is evident in DR-*rd10* compared to LR-*rd10* (Fig. 18.2c).

18.3.3 QrtPCR Analysis of Candidate Retinal Genes

While rhodopsin expression is lost by PNM2.5 in LR-*rd10* mice, the expression in DR-*rd10* is maintained at 79% of WT levels. DR-*rd10* *PDE6β* expression levels are also close to WT.

GS expression in Müller glia, possibly indicative of a continued retinal response to glutamate, is found to be maintained in both LR-*rd10* and DR-*rd10* mice.

18.4 Discussion

Light restriction has been shown to slow degenerations in 15 of 20 rodent strains surveyed (reviewed by Paskowitz et al. 2006; Valter et al. 2009). Particularly robust findings have been documented in the RCS rat (Dowling and Sidman 1962; Kaitz and Auerbach 1979), the P23H-3 transgenic mouse (Naash et al. 1996) and rat (Bicknell et al. 2002), and in the rhodopsin-mutant dog (Cideciyan et al. 2005). However, it is in the *rd10* mouse that the protective effect of dark-rearing is most evident (Chang et al. 2007). An effort is made in this preliminary study to characterize this effect and improve our understanding of the nature of the *rd10* mutation. A therapeutic strategy that takes advantage of this protective effect may be translated to human patients with photoreceptor-based RD mutations. Recently, it has been demonstrated that bipolar cells of the *rd10* retina undergo histochemical and morphological changes during the course of degeneration with loss of nearly a quarter of RBCs and a differential loss of responsiveness to glutamate (Gargini et al. 2007; Puthussery et al. 2009). A survival strategy for those RBCs that remain may be observed in the extension of processes to the remaining cones of the ONL (Puthussery et al. 2009). Our results imply that the protective effects of dark-rearing are a consequence of altered synaptic contacts in the OPL that preserved function of both the ONL and INL. Significant preservation of rod photoreceptor and RBC function is found in DR-*rd10* mice compared to LR-*rd10* mice until PNM3 (Fig. 18.1) and OPL synaptic contacts are more prevalent in the DR *rd10* than in the age-matched LR-*rd10* (Fig. 18.2). The normal scotopic b-wave in ERG suggests that these RBC-photoreceptor synapses are functional.

In the *rd/rd* retina, it has been hypothesized that the RBCs do not receive sufficient input from healthy photoreceptors to enable normal development of glutamate signaling pathways (Chua et al. 2009). In the case of the *rd10* model, it may be the *maintenance* of glutamate signaling pathway that is compromised, as the inner retina somehow succumbs to secondary effects of the *PDE6 β* mutation. As the DR-*rd10* is never challenged with light, the undisrupted glutamate flow may help maintain downstream signaling pathways. This initial investigation offers evidence of the extent of this protective effect. It suggests that appropriately timed light restriction at the time of accelerated degeneration may help support retinal circuitry prior to therapy and maximize the chance of a positive outcome. Alternatively, it may be possible to address the primary cause of retinal degeneration in this model – such as alterations in glutamate release/replacement. Evidently, further investigation is needed before the exact nature of the dark-rearing protective effect can be understood in the *rd10* mice.

Up to 21 mutations have been found within the *PDE6 β* catalytic domain in patients with recessive RP (<http://www.hgmd.cf.ac.uk/>). This preliminary study into long-term maintenance of rod function in the dark-reared *rd10* mouse suggests that approaches to maintain rod function may be extrapolated to human patients.

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Fig. 18.1.

Time course of changes in the ERG in *rd10* mice: no decline at least in the first 2 months

Fig. 18.2. Immunolabelling of LR-rd10 vs. DR-rd10 mice at PNM2.5. (a) PNA (red) and PKC α (green) expression. (b) VAMP-2 and (c) Synapsin-1 Scale bar, 50 μ M

Fig. 18.3.
Retinal gene expression in light-reared (LR) vs. dark-reared (DR) *rd10* mice at PNM2.5