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Photoreceptors in *whirler* mice show defective transducin translocation and are susceptible to short-term light/dark changes-induced degeneration

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ABSTRACT

Usher syndrome combines congenital hearing loss and *retinitis pigmentosa* (RP). Mutations in the whirlin gene (*DFNB31/WHRN*) cause a subtype of Usher syndrome (USH2D). *Whirler* mice have a defective whirlin gene. They have inner ear defects but usually do not develop retinal degeneration. Here we report that, in *whirler* mouse photoreceptors, the light-activated rod transducin translocation is delayed and its activation threshold is shifted to a higher level. Rhodopsin mis-localization is observed in rod inner segments. Continuous moderate light exposure can induce significant rod photoreceptor degeneration. *Whirler* mice reared under a 1500 lux light/dark cycle also develop severe photoreceptor degeneration. Previously, we have reported that *shaker1* mice, a USH1B model, show moderate light-induced photoreceptor degeneration with delayed transducin translocation. Here, we further show that, in both *whirler* and *shaker1* mice, short-term moderate light/dark changes can induce rod degeneration as severe as that induced by continuous light exposure. The results from *shaker1* and *whirler* mice suggest that defective transducin translocation may be functionally related to light-induced degeneration, and these two symptoms may be caused by defects in Usher protein function in rods. Furthermore, these results indicate that both Usher syndrome mouse models possess a light-induced retinal phenotype and may share a closely related pathobiological mechanism.

1. Introduction

Usher syndrome is a clinically and genetically heterogeneous disease. It is the most common cause of combined sensorineural hearing impairment and *retinitis pigmentosa* (RP) (Smith et al., 1994). In some cases, vestibular dysfunction (Hallgren, 1959) and mental disturbances (Boughman et al., 1983) are also symptoms of the syndrome. Three major clinical types of Usher syndrome (type I, II, and III) can be distinguished based on the severity and progression of hearing loss and the age of onset of RP. Currently, ten different genes are known to be associated with the various subtypes of Usher syndrome (Williams, 2008; Kremer et al., 2006; Reiners et al., 2006; Saihan et al., 2009; Riazuddin et al., 2012). However, even though there are reports about the ability of several of these Usher proteins to form complex through molecular interaction in photoreceptors (Maerker et al., 2008; Van-Wijk et al., 2006; Yang et al., 2010), the disease mechanism of RP in Usher syndrome remains unknown.

Mutations in the *DFNB31/WHRN* gene, which encodes a protein called whirlin (Mburu et al., 2003), cause a subtype of Usher syndrome, type IID (USH2D) (Ebermann et al., 2007). In vertebrate retina, whirlin protein is expressed in the photoreceptor cells. In the photoreceptors, whirlin protein accumulates at cilium region and synaptic terminals (Kersten et al., 2010; Maerker et al., 2008; Van-Wijk et al., 2006; Yang et al., 2010). *Whirler* mice have mutations in *DFNB31/WHRN* gene and are an accepted animal model for USH2D. *Whirler* mice have auditory dysfunction, and their cochlear hair cells have abnormally formed stereocilia (Holme et al., 2002). However, like several other naturally occurring Usher mouse models, *whirler* mice do not develop retinal degeneration (Mburu et al., 2003).

Previously, we have reported that the rod photoreceptors in *shaker1* mice, a well-accepted mouse model for USH1B, showed delayed rod transducin translocation with a shift of its light

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activation threshold to a significantly higher level (Peng et al., 2011). In rod photoreceptors, it has been suggested that the transducin translocation activated by a specific light threshold, may serve as a neuroprotective function for rods under conditions of high intensity light by reducing metabolic stress (Artemyev, 2008; Calvert et al., 2006; Kalra et al., 2007; Lobanova et al., 2007; Sokolov et al., 2002; Slepak and Hurley, 2008). Indeed, we have found that continuous exposure of *shaker1* mouse under even moderate intensity light could induce significant rod photoreceptor degeneration. Furthermore, when *shaker1* were reared under a moderate light (1500 lux/dark cycle), they develop severe retinal degeneration in less than 6 months (Peng et al., 2011). We have further observed that subretinal injection of wild type myosin VIIa could rescue both light-induced degeneration and delayed transducin translocation, indicating these symptoms are caused by defects in myosin VIIa (Zallocchi et al., 2011).

Here, we report that, similar to *shaker1* mice, the rod photoreceptors in *whirler* mice also show delayed transducin translocation with a shift of its light activation threshold to a significantly higher level and sensitivity to moderate light-induced photoreceptor degeneration. In addition, similar to previous reports for *shaker1* mice (Liu et al., 1999), *whirler* mice show immunostaining for rhodopsin in the inner segments, suggesting a possible rhodopsin mis-localization. Interestingly, we have found that, alternative short-term 1 h moderate light exposure with 7 h dark adaptation induces photoreceptor degeneration in both *shaker1* mice and *whirler* mice as severe as that induced by continuous light exposure. These light conditions do not affect strain/age matched wild type retinas. Our findings from these two mouse models indicate a clear connection between defective transducin translocation and light-induced degeneration. These results also show that, similar to *shaker1* mice, *whirler* mice do indeed possess a robust retinal phenotype, which has likely been missed due to dim light conditions in most animal vivariums. More importantly, these results show that these two Usher syndrome mouse models, alluding to a closely related pathobiological mechanism.

2. Methods

2.1. Ethics statement

All animal handling and procedures were performed in accordance with protocols for these studies that have been approved by the Boys Town National Research Hospital Institutional Animal Care and Use Committee (IACUC) and in accordance with NIH and USDA guidelines. Every effort was made to minimize discomfort and distress.

2.2. Animals

Pigmented *whirler* mice (*B6.Cg-Whrn^{wi}Tyrp1^{b}/++/J*) were purchased from Jackson Laboratories (Bar Harbor, ME; Stock Number: 000571). These mice harbor a 526 base pair deletion abolishing the short C-terminal isoform and creating a frame shift that results in premature termination of the long isoform before the third PDZ domain (Mburu et al., 2003). Pigmented 129 Sv/J mice were used as wild type control mice. *Whirler* mice were back-crossed onto the pigmented 129 Sv/J background seven times for purposes of comparison with the wild type mice. In both *whirler* mice and control wild type mice the RPE65 transcript was amplified and sequenced and found to be of the L450 genotype (Danciger et al., 2000; Wenzel et al., 2001), therefore, both *whirler* and the wild type mice used in these studies harbor the L450 quantitative trait locus for RPE65, and their photoreceptors are thus inherently more sensitive to light induced damage than the mice with L450M in their RPE65, which are much less sensitive to light induced photoreceptor damage (Danciger et al., 2000; Redmond et al., 2007; Wenzel et al., 2001). Thus, under the same light conditions, the results may not be reproduced used in strains that are L450M for RPE65, which would have much higher resistance to light damage. The animals were kept in transparent cages under 12 h light/dark cycle at the Boys Town National Research Hospital (BTNRH) *vivarium*. Procedures for light/dark adaptation did not cause pain, discomfort, distress or morbidity. The animals were anesthetized with a mixture of ketamine 300 mg/kg and xylazine 30 mg/kg body weight, administered IP, prior to euthanizing by cervical dislocation to eliminate the potential for pain. Tissues were obtained after the animals were euthanized.

2.3. Antibodies

Antibodies against the following proteins were used: rhodopsin (Sigma, MO), centrin1 (Santa Cruz, CA), cytochrome C (Millipore, MA) and α subunit of rod transducin (CytoSignal, CA). Anti-R9AP antibody was a gift from Dr. V. Arshavsky, Duke University.

2.4. Light/Dark adaptation

Light and dark adaptation, including continuous 6 day moderate light exposures and long term moderate light/dark cycle were performed exactly as described in an earlier paper where *shaker1* mice were analyzed (Peng et al., 2011). For alternative short-term light/dark adaptation changes, the animals were first kept in transparent cages without any restraint in a lightproof darkroom for 8 h dark adaptation, and then the animals were first exposed under 2000 lux light for 1 h. Diffuse white fluorescent light were placed 4–6 inches above the cages and beside the cages on all four sides. Light intensity was measured inside the cage. After this one hour light exposure animals were kept in a darkroom for dark adaptation for 7 h, and then the mice were exposed again to 2000 lux light for 1 h. After this, the mice were dark adapted for 7 h again. Such 2000 lux 1 h-light exposure/7 h-dark adaptation alternative changes were repeated for 2 weeks.

2.5. Immunocytochemistry

Methods used for immunohistochemical analysis have been described in detail in previous publications (Peng et al., 1997, 2000, 2003, 2011). Briefly, mouse eyes were removed from euthanized animals, and were fixed in 4% paraformaldehyde in 100 mM sodium phosphate buffer (PB, pH 7.3) at 4 °C. The tissue was then transferred into 5% sucrose in PB at 4 °C overnight and then 30% sucrose in PB at 4 °C overnight. Retinal sections, cut with a Microm cryostat and mounted on gelatin-coated slides, were incubated first with 5% normal goat serum (Vector Laboratories) in PBS for 1 h at room temperature, and then incubated with primary antibodies overnight at 4 °C [anti-transducin α1 antibody 1:1000; anti-rhodopsin antibody 1:1000; anti-R9AP 1:1000], followed by three washes in PBS. All incubation and wash buffers contained Triton X-100 (0.3%). The sections were then incubated with Alexa 594-conjugated anti-mouse immunoglobulin antibody or Alexa 488-conjugated anti-rabbit immunoglobulin antibody (Invitrogen, Eugene, OR) 1:250 for 2 h. For double-immunostaining, retinal sections were incubated with mixed primary and secondary antibodies. The slides were then washed with PBS and coverslipped with 50% glycerol in PBS for viewing under a Zeiss AX10 microscope.

2.6. Serial tangential sectioning and western blotting

The method is modified based on that described by Sokolov et al. (2002)), and Martemyanov et al. (2003). After dark