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FUNCTIONAL AND STRUCTURAL MODIFICATIONS DURING RETINAL DEGENERATION IN THE rd10 MOUSE

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Abstract—Mouse models of retinal degeneration are useful tools to study therapeutic approaches for patients affected by hereditary retinal dystrophies. We have studied degeneration in the rd10 mice both by immunocytochemistry and TUNEL-labeling of retinal cells, and through electrophysiological recordings. The cell degeneration in the retina of rd10 mice produced appreciable morphological changes in rod and cone cells by P20. Retinal cell death is clearly observed in the central retina and it peaked at P25 when there were 800 TUNEL-positive cells per mm^2. In the central retina, only one row of photoreceptors remained in the outer nuclear layer by P40 and there was a remarkable deterioration of bipolar cell dendrites postsynaptic to photoreceptors. The axon terminals of bipolar cells also underwent atrophy and the inner retina was subject to further changes, including a reduction and disorganization of All amacrine cell population. Glutamate sensitivity was tested in rod bipolar cells with the single cell patch-clamp technique in slice preparations, although at P60 no significant differences were observed with age-matched controls. Thus, we conclude that rod and cone degeneration in the rd10 mouse model is followed by deterioration of their postsynaptic cells and the cells in the inner retina. However, the functional preservation of receptors for photoreceptor transmission in bipolar cells may open new therapeutic possibilities.

Key words: retina, rod, cone, bipolar cells, phosphodiesterase, apoptosis.

Retinitis pigmentosa (RP) includes a large group of inherited retinal disorders that cause progressive loss of retinal function. Indeed, it represents one of the main causes of blindness in the world, with an incidence of approximately one in 4000 humans (Berson, 1993). Approximately one half of RP patients have a mutation in genes that encode proteins involved in the phototransduction cascade. Mutations in the gene encoding the β subunit of the cGMP phosphodiesterase (β-PDE) have been identified in patients with autosomal recessive RP (McLaughlin et al., 1993), which account for approximately 5% of such cases in the world. The identification of these mutations has facilitated the development of mouse models of retinal degeneration, which have been used to study the cellular and molecular mechanisms of photoreceptor degeneration (reviewed in Chang et al., 2002).

The rd1 mouse is one of the best known models of retinal degeneration (*Pdeb*$^{rd1-/rd1-}$, for review see Farber et al., 1994). In this model, a point mutation in exon 7 of the β-PDE gene leads to the rapid, large-scale loss of rod photoreceptors within 15–20 days of postnatal life (P15–20, Portera-Cailliau et al., 1994). Despite this rapid degeneration, the rd1 model has been widely used to study the mechanisms underlying retinal degeneration (Doonan et al., 2003; Hart et al., 2005). The structural modifications to retinal neurons following photoreceptor degeneration have been characterized in rd1 animals (Marc et al., 2003; Jones and Marc, 2005; Strettoi and Pignatelli, 2000; Strettoi et al., 2003), and the sensitivity of degenerated rod postsynaptic retinal cells to retinal neurotransmitters has also been addressed in this model (Varela et al., 2003). Accordingly, the rd1 mouse model of retinal degeneration has been used to test therapeutic approaches that may inhibit the process of photoreceptor apoptosis and degeneration (Frasson et al., 1999a,b; Takano et al., 2004; Komeima et al., 2006; MacLaren et al., 2006).

Other animal models used to study how progressive photoreceptor degeneration affects rod and cone relay pathways include a rat model with a recessive mutation generated by the Royal College of Surgeons (RCS), in which the gene encoding for the Mertk tyrosine kinase receptor is disrupted (D'Cruz et al., 2000; Dufour et al., 2000). In this rat model, retinal pigment epithelial cells fail to phagocytose the rod outer segments shed, leading to the generation of a zone of debris of outer segment material and progressive photoreceptor degeneration (Cuenca et al., 2005). The P23H transgenic rat is a model of autosomal dominant RP, in which a mutation in the rhodopsin gene leads to a loss of rods and to a more protracted loss of cones (Cuenca et al., 2004). In both animal models, there is substantial loss of rods and a reduction of rod bipolar dendrites by P21. During the course of photoreceptor loss, the progressive changes mainly affect cells that are involved in the rod relay pathway. By P60, a few cells still remain in the outer nuclear layer, whereas in the rd1

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Abbreviations: ERG, electroretinogram; IPL, inner plexiform layer; mGluR6, metabotropic glutamate receptor 6; P, postnatal day; RCS, Royal College of Surgeons; RP, retinitis pigmentosa; β-PDE, β subunit of the cGMP phosphodiesterase.

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mouse the rods begin to degenerate around P8–P10, and by P20 only a few scattered photoreceptors are left (Strettoi et al., 2002).

Recently, another mouse model with a spontaneous mutation was described, the rd10 strain (Chang et al., 2007; Gargini et al., 2007). The rd10 mouse strain is a newly described model of retinal degeneration that bears a point mutation in exon 13 of the β-PDE gene (Chang et al., 2002, 2007). In the rd10 mouse, the specific mutation in β-PDE also leads to a massive loss of rod photoreceptors in the first weeks of postnatal life, although the time course of retinal degeneration seems to be slower than in the rd1 mouse. Morphological and physiological studies (Gargini et al., 2007) have shown that the rd10 mouse model may be a good model to assess rescue approaches, since photoreceptor degeneration starts around P18. The delay of rod death in the rd10 mouse means that retinal degeneration does not coincide with the period of photoreceptor maturation, as is the case in the rd1 model. The time course of retinal degeneration in rd10 has been described (Gargini et al., 2007) and it starts at P18 peaking around P25. Furthermore, the secondary degeneration experienced by bipolar and horizontal cells postsynaptic to degenerated rods was also studied. Indeed, electroretinogram (ERG) studies revealed that the alteration in the physiology of the inner retina parallels the structural degeneration.

To gain insight into the progress of photoreceptor degeneration and the associated secondary events, we carried out structural and electrophysiological experiments in the rd10 mouse model of rod degeneration (*Pdeb*$^{rd10-/rd10-}$). Here, we have characterized several aspects of rd10 retinal degeneration that have not yet been addressed. We have counted the degenerating photoreceptors, which allowed us to estimate the number of photoreceptors undergoing degeneration at different times. Moreover, we studied cone and rod morphology during retinal degeneration to gain an idea of the ability of cone cells to survive rod degeneration. During this period, we also studied modifications in the connectivity between rods, cones and their postsynaptic bipolar and horizontal cells. Indeed, our structural study looked further into the inner retina to describe the morphological alterations experienced by cells in the rod pathway. Finally, to test whether rod degeneration determines functional modifications in inner retinal cells, we studied the effect of retinal neurotransmitters on bipolar cells postsynaptic to degenerated rods. These studies complete the information regarding the rd10 mouse model of retinal degeneration (Chang et al., 2007; Gargini et al., 2007), providing a more complete morphological and functional characterization of this rd10 model and highlight the benefits of using this model in future research.

EXPERIMENTAL PROCEDURES

Animals

C57BL/6j (wild type) and C56BL/6j$^{rd10/rd10}$ (rd10) mice were used in the experiments at P20, P25, P30, P35, P40 and P60. All animals were obtained from the Jackson Laboratory (Bar Harbor, ME, USA), and they were maintained and bred at the University of Alcalá on a 12-h light/dark cycle. Light cycle illumination of 30 cd/m^2 was measured with a photometer (Mavo Monitor USB, Gossen, Nürnberg, GE). All animals were handled in accordance with the European Union guidelines for the use of laboratory animals (European Directive 86/609/EEC), minimizing animal suffering and numbers used for experiments.

ERG recordings

Prior to ERG recording, mice were adapted to the dark overnight. The mice were then anesthetized under dim red light by i.p. injecting a solution of ketamine (95 mg/kg) and xylazine (5 mg/kg) and they were then maintained on a heated pad at 37 °C. The pupils were dilated by topical application of 1% tropicamide (Colircusí Tropicamida, Alcon Cusí, SA, El Masnou, Barcelona, Spain) and to optimize electrical recording, a topical drop of 2% Methocel (Ciba Vision AG, 8442 Hetlingen, Switzerland) was instilled in each eye immediately before the corneal electrode was put in place. Anesthetized animals were placed on a Faraday cage and all experiments were performed in absolute darkness. The eyelids of the mice were separated to optimize electrode application and light stimulation. Flash-induced ERG responses were recorded from the right eye in response to light stimuli produced with a Ganzfeld stimulator. The intensity of the light stimulus was measured with a photometer (Mavo Monitor USB) at the level of the eye and for each intensity of light, an average of 4 to 64 consecutive light stimuli was presented. The interval between light flashes applied in scotopic conditions was 10 s for dim flashes and up to 60 s for the highest intensity. Under photopic conditions, the interval between light flashes was fixed at 1 s. The ERG signals were amplified and band filtered between 0.3 and 1000 Hz with a Grass amplifier (CP511 AC amplifier, Grass Instruments, Quincy, MA, USA). Electrical signals were digitized at 10 kHz with a Power Laboratory data acquisition board (ADI Instruments, CA, USA). Recordings were saved on a PC and analyzed off line. Bipolar recordings were obtained using an Ag:AgCl mouse electrode fixed on a corneal lens (Burian-Allen electrode, Hansen Ophthalmic Development Laboratory, Coralville, IA, USA), a reference electrode located in the mouth, and with a ground electrode located on the tail. The electrode was mounted on a coarse micromanipulator for easy positioning over the mouse eye.

Rod-mediated responses were recorded under dark adaptation following light flashes ranging from -4 to -1.52 log cd·s·m^{-2}. Mixed rod- and cone-mediated responses were recorded following light flashes ranging from -1.52–0.48 log cd·s·m^{-2}. Cone-mediated responses were recorded following light flashes ranging from -0.52–2 log cd·s·m^{-2} on a rod saturating background of 30 cd/m^2. The amplitude of the a-wave was measured from the baseline to the trough of the a-wave and the results were averaged. Likewise, the amplitudes of the b-wave were measured from the trough of the a-wave to the peak of the b-wave and averaged. Measurements were recorded by an observer who was blind to the experimental condition of the animal and the statistical analysis was performed using the Student's *t*-test.

TUNEL-labeling of whole-mounted retinas

Animals were decapitated at P20, P25, P30, P35 and P40, their eyes were removed and the retinas were dissected out and flat-mounted on nitrocellulose filter (Sartorius, Göttingen, Germany) with the photoreceptor layer side up. The retinas were then fixed in 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 at 4 °C overnight. To detect apoptotic cells, whole mounted retinas were processed for TUNEL (Promega, Madison, WI, USA) according to the manufacturer recommendations. After labeling, the retinas were counterstained with DAPI and mounted in vectashield (Vector Laboratories, Burlingame, CA, USA) for microscopical observation. A TCS SP2 Laser-confocal microscope (Leica, Microsystems, Wetzlar,