Extended Photoreceptor Viability by Light Stress in the RCS Rats but not in the Opsin P23H Mutant Rats

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PURPOSE. To determine the effect of light stress on retinal function and long-term photoreceptor viability in Royal College of Surgeons (RCS) rats and the applicability of the light treatment to the ops in P23H mutant rats.

METHODS. RCS rats at postnatal day (P)23 were illuminated with 120 foot-candles (fc) white light for 10 hours. Photoreceptor survival and basic fibroblast growth factor (bFGF) expression were measured at P60 and P83. Retinal function was evaluated by electroretinography. Opsin P23H transgenic rats were treated with light at P28 and analyzed at P70 for photoreceptor viability, ultrastructure, and bFGF expression.

RESULTS. Light-treated RCS rats at P60 had four to five rows of nuclei versus one to two rows in untreated littermates. The average amplitude of the ERG b-wave was 28 µV in treated rats, compared with 6 µV in untreated littermates. By P83 there was still significant preservation of the ONL in treated rats. Immunoblot analysis showed a high expression of bFGF in the treated retinas even 2 months after treatment. Illumination of P23H rats at P28 with 120 fc white light for 10 hours caused substantial photoreceptor cell death, although bFGF expression was upregulated. Lowered illumination dosages continued to cause photoreceptor damage until levels were reached that neither caused damage nor enhanced survival.

CONCLUSIONS. Although light stress promotes photoreceptor survival and function in the RCS rat, it elicits death signals in the P23H rats that may not be overcome by survival-promoting factors. Therefore, use of light stress to promote photoreceptor survival should be considered with regard to sensitivity of the mutation to light damage. (Invest Ophthalmol Vis Sci. 2001;42:842–849)

Exposure of the retina to intense visible light elicits a series of reactions leading to apoptotic photoreceptor cell death.¹ ² The severity of the damage is determined by the light’s intensity, length of exposure, and wavelength.³ ⁵ ⁷ ⁸ Recently, it has been recognized that in addition to its damaging effect, exposure to intense light also evokes a response that protects photoreceptors. By using a preconditioning paradigm, it has been shown that exposure to intense light for a short period protects photoreceptors from subsequent exposure to damaging light.⁶ Because exposure to light stimulates the expression of basic fibroblast growth factor (bFGF) in the retina,⁷ ⁸ the protection induced by light could be attributed to elevated levels of bFGF which are measured in the light-stressed retinas.⁶ Direct involvement of bFGF in protection of photoreceptors was demonstrated in studies in which intraocular injections of bFGF reduced photoreceptor cell death due to excessive light.⁹ ¹⁰ ¹¹

In a previous study, we showed that light treatment enhances photoreceptor survival in dystrophic retinas of Royal College of Surgeons (RCS) rats. Illumination with 12 hours of bright light at postnatal day (P)23 resulted in the retention of numerous photoreceptor nuclei at P42. Elevated expression of bFGF was measured in the treated rats.¹² These findings extend the light–rescue paradigm to the retinal degeneration that is caused by a genetic mutation. In the present study, we explored the functional significance of enhanced survival in RCS rats, evaluated by electroretinogram (ERG), and the long-term effect of light treatment on photoreceptor survival. To evaluate the applicability of light treatment to a different animal model of retinal dystrophy, the transgenic P23H rat was investigated.

The RCS rat is characterized by reduced capacity of the pigment epithelium to phagocytose shed rod outer segment tips. The phagocytic malfunction results in accumulation of membranous debris in the subretinal space and subsequently to apoptotic photoreceptor cell death and blindness.¹³ ¹⁴ ¹⁵ The mutation in the RCS rat was recently localized to a receptor tyrosine kinase merck gene.¹⁶ The P23H transgenic rats carry a point mutation in the opsin gene in which histidine in position 23 is replaced by proline.¹⁷ ¹⁸ ¹⁹ The mutation leads to progressive loss of photoreceptors and to blindness, possibly due to misrouting of transduction proteins or defective disc membrane morphogenesis.²⁰ ²¹ In humans, the P23H mutation is the most common mutation found in patients with autosomal dominant retinitis pigmentosa.²²

Mutant rats were treated with light at a postnatal age before onset of measurable degenerative cell death. Photoreceptor survival, bFGF expression, and retinal function were evaluated. Although light treatment significantly enhanced photoreceptor survival and function in RCS rats, light stress negatively affected photoreceptors in the P23H retinas. Therefore, the potential use of light therapy to extend photoreceptor survival in dystrophic retinas should be selective. Appropriate animal models for the specific genotype should be evaluated to determine the consequences of the light treatment.

METHODS

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Animals

Tan-haired, pink-eyed RCS rats²³ and transgenic P23H rats²⁴ (line 3, kindly provided by Matthew M. LaVail, University of California, San Francisco) were raised at the local animal facility. The rats were maintained under a diurnal cycle of 12 hours dark and 12 hours light with an illumination intensity of 3 foot-candles (fc; 1 fc = 10.76 lux). Normal Sprague–Dawley rats (Harlan, Indianapolis, IN) were kept under the same illumination conditions for at least 2 weeks before experimentation.

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Light Stress

Bright white light was produced by two fluorescent lamps, 40 W each (Cool White; General Electric, Fairfield, CT). Illuminance intensities were determined by the distance of the lamps from the cage floor. In some experiments, the light was filtered through a green sheet of Plexiglas (number 2092; DuPont, Wilmington, DE) to produce transmission. The rats were anesthetized with intraperitoneal ketamine (75 mg/kg) and xylazine (10 mg/kg). Pupils were dilated with 1% tropicamide. The corneas were kept moist with 2.5% hydroxypropyl methylcellulose. The rats were placed prone on a catalytic heating pad (Deltech Isothermal Pad; Braintree Scientific, Braintree, MA). ERGs were recorded with an active carbon fiber electrode on the cornea, an indifferent gold ring electrode on the tongue, and a ground platinum needle subcutaneous electrode near the tail. The rat eye was centered in the aperture of the Ganzfeld (model 2505; LKC Technologies, Gaithersburg, MD). Bioelectric signals were amplified with a gain of 10,000 and filtered from 1 to 1000 Hz (model P5 amplifier; Grass, Quincy, MA). The amplified signals were digitized, recorded, and averaged by a digital oscilloscope (model 4094 B; Nicolet, Madison, WI). The signals were digitized at 10,000 samples/sec with 12-bit analog-to-digital resolution in a trace of 0.396 seconds’ duration. The flash stimulus source (model PS22; Grass) that evoked the ERG was seen by diffuse reflection off the interior surface of the Ganzfeld. Flash luminance was 9 scotopic candelas (cd/sec²) (model 1700 with SED035 detector and ZCIE scotopic filter, International Light, Newburyport, MA). The rats were dark adapted a further 10 minutes after preparation. Two ERGs were recorded with a minimum interval of 10 seconds. The b-wave amplitude was measured from the preceding trough to the peak of the b-wave.

RESULTS

Photoreceptor Survival and ERG Measurements in Light-Treated RCS Rats

Animals were treated with a single dose of 120 fc light for 10 hours at P23 and analyzed at P60, an age by which major cell loss occurred in untreated RCS rats. A significant increase in photoreceptor survival was noted in the 3-mm length of retina from the optic nerve head to the midperiphery. In the far periphery, a region approximately 1 mm from the ora serrata, protection by light treatment was very limited. The most consistent levels of ONL width were measured at a distance of approximately 1 to 2 mm from the optic nerve head. The results of quantitative analysis of the ONL width in this region are presented in Figure 1. The average ONL width of light-treated retinas in this area was 16.73 ± 1.13 μm (n = 10), whereas in the untreated retinas the same area measured 2.04 ± 0.3 μm (n = 7), an eightfold difference. In normal Sprague-Dawley rats at P56 to P66, the ONL width in the posterior retinas measured 41.7 ± 1.4 μm (n = 4). Thus, approximately 40% of the normal ONL width is preserved in the light-treated RCS retina in the region 1 to 2 mm away from the optic nerve head. Examination of serial sections of treated retinas revealed that the ONL survival extended to all quadrants of the retina.

As shown in Figure 2A, the light-treated rats at P60 had a relatively well-preserved ERG b-wave with prominent oscillatory potentials on the ascending slope of the b-wave. The ERG
of the untreated littermates was typical of dystrophic RCS rats and exhibited a barely detectable b-wave with no oscillatory potentials (Fig. 2B). The within-animal variability of the ERG data was very low, as illustrated by the superimposed repeated ERGs of the light-treated and untreated rats (Figs. 2A, 2B). Data from eight light-treated and seven untreated RCS rats are illustrated in Figure 2C. There were larger b-wave amplitudes in all treated rats than in untreated littermates. The average b-wave amplitude in treated retinas was 28 μV versus 6 μV in untreated retinas. The mean difference in b-wave amplitudes in the two groups was significant by a two-tailed t-test with a Welch correction for the difference in variability (t = 4.62, P < 0.01). Considerable variability was measured in b-wave amplitude among the eight light-treated RCS rats, possibly reflecting different levels of photoreceptor preservation as revealed by measurements of ONL width (Fig. 2C). For comparison purposes, b-wave amplitude in Sprague–Dawley retinas under the same stimulus conditions was 625 μV.

Long-Term Photoreceptor Survival in Light-Treated RCS Rats

The end point of photoreceptor degeneration in the retina of the pink-eyed RCS rat occurs between P80 and P90. We investigated the long-term effect of light treatment in RCS rats at P83, 2 months after exposure to a single dose of intense light. This selected experimental period of P23 to P83 also correlates with the period used in a study in which application of exogenous bFGF was found to protect photoreceptors in the RCS rat.11 By P83, the ONL in the retinas of untreated littermates was completely absent in some areas or reduced to a few pyknotic nuclei in a single row in other areas (Fig. 3A). The retinas of animals treated with light, however, contained areas that had a considerable number of normal photoreceptor nuclei. The surviving nuclei were unevenly distributed along the posterior periphery axis of the retina. Whereas some sites were devoid of nuclei, others had two to four rows of nuclei (Fig. 3B). Ultrastructural analysis of surviving nuclei in light-treated retinas revealed normal morphologic characteristics (Fig. 3C). Because of the great variation in the number of surviving photoreceptor nuclei at various sites in the retina, measurements of ONL width at given intervals produced great variation in values. To reliably reflect the extent of increase in photoreceptor nuclei survival, the number of nuclei along a 100-μm segment of the retina was counted in light-treated and untreated retinas (Fig. 4). For comparison, the number of nuclei in light-treated and untreated retinas at P60 are included (Fig. 4). Substantial variations in number of nuclei between different retinas and between the two hemispheres of the same retina were observed. However, the number of remaining nuclei in the treated retinas was much larger than in the untreated control retinas.

For further quantitative assessment, the total number of nuclei was counted along the full length of a 1-μm-thick section, which included the complete length of the retina from...
the optic nerve head to the ora serrata, a distance of approximately 4 mm. Superior and inferior hemispheres along the vertical meridian were analyzed in 14 eyes of treated rats. Four eyes of untreated littermates were used as control retinas. In treated retinas, 665.6 ± 45 (mean ± SD; n = 14) nuclei per section were counted, whereas in untreated retinas the number was 186.1 ± 35.3 (n = 4). As noted, all the remaining nuclei in untreated retinas were highly pyknotic. Thus, by P83 there were 3.5 times more photoreceptor nuclei in retinas treated with a single dose of intense light 2 months earlier at P23. The ERG of two treated RCS rats at P83 showed b-wave amplitudes of 9.6 and 9.3 μV, whereas an untreated control RCS rat at P83 had a b-wave amplitude of 2.2 μV. Thus, the survival of photoreceptor nuclei with normal morphology in light-treated RCS rats at P83 was reflected by the functional measurements.

bFGF Protein Expression in Light-Treated RCS Retinas at P60 and P83

For analysis of bFGF protein expression, retinas were collected at P60 and P83 from RCS rats that had been treated with a single exposure of 120 fc light for 10 hours at P23. Retinas of untreated littermates served as control specimens. Immunoblot analyses revealed a significant increase in bFGF protein in the treated retinas at both P60 and P83. At P60 (Fig. 5A), there was a 40% increase in the 18-kDa bFGF. A higher increase of 102% in the sum of the two high-molecular-weight forms (24 and 22.5 kDa) of bFGF was also seen. By P83, there was still a significant upregulation of bFGF (Fig. 5B). Light-treated retinas had a 42% increase in the 18-kDa bFGF protein and a 50% increase in the sum of the two high-molecular-weight forms of bFGF.

Photoreceptor Viability in Light-Treated Transgenic P23H Rats

In P23H (line 3) rats, the ONL is reduced to four to seven rows of nuclei by P60. In preliminary studies, we did not find measurable photoreceptor cell loss before P30. Therefore, to evaluate the effect of light stress, P23H rats were treated at P28 and analyzed at P70. In the first set of experiments, the same protocol as was applied to RCS rats was used with P23H rats. Animals were illuminated for 10 hours with 120 fc of bright light. Unlike the results in RCS rats, illumination of P23H rats at P28 with bright light for 10 hours caused substantial photoreceptor cell loss in all regions of the retina at P70, when compared with untreated rats. In Figure 6, the ONL width in control (Fig. 6A) and light-treated (Fig. 6B) P23H rats at P70 is depicted. The posterior region of the superior retina where the light damage was more pronounced is shown. Whereas the ONL in untreated retina contained five to six rows of nuclei, it was reduced to three to four in the light-treated retinas, indicating light-mediated photoreceptor cell death. Ultrastructural analysis of light-treated retinas and untreated P23H retina is shown in Figures 6C and 6D. In the untreated retina at P70, intact outer and inner segments were observed. In the light-treated retina, shortened malformed outer segments and inner segments were seen. Only four rows of photoreceptor nuclei remained in the observed site.

In view of the considerable light damage caused by 10 hours’ illumination at 120 fc, subsequent experiments with lower levels of illumination were performed. However, considerable reduction in illumination levels and duration continued to produce light-damage in the P23H retina. Quantitative analysis of ONL width after different light treatments is presented in Figure 7. ONL width in untreated normal Sprague-Dawley rats at P55 through P65 and in untreated P23H rats at P70 was compared with that in light-treated P23H rats at P70.
A 33% reduction in ONL width in the untreated P23H rats, compared with normal Sprague–Dawley rats, is a measure of the degenerative photoreceptor cell loss in the P23H rat retina. A 34.5% reduction in ONL width in P23H rats treated with 120 fc for 10 hours (at P28) is a measure of light-mediated photoreceptor cell death (in addition to degenerative cell death). Lowering the illumination dosage to 50 fc for 8 hours produced a 19% reduction in ONL width in comparison with untreated P23H retinas. Further reduction of illumination levels to 50 fc for 4 hours and use of a green filter to eliminate possible damaging short wavelength did not prevent light damage. Finally, reduction of light dosage to 0.5 to 1 hour at 120 fc had a 19% reduction in ONL width in comparison with untreated littermates (data not shown). Thus, unlike the RCS rats, the P23H rats are highly susceptible to light rescue could not be obtained.

FIGURE 5. Expression of bFGF at P60 and P83 in RCS retinas treated with bright light (120 fc) for 10 hours at P23. The amount of bFGF proteins was determined by immunoblot analysis. Retinas were collected at P60 and P83 from animals that had been treated with a single dose of 10 hours of bright light at P23. Each lane represents a sample of 100 μg total protein from two retinas of the same animal. Proteins of bFGF were detected as three distinct bands of 24, 22.5, and 18 kDa. (A) P60: Data for each band were averaged from lanes 1 through 4 (untreated control retinas) and lanes 5 through 8 (light-treated retinas). The amount of the 18-kDa form of bFGF protein was 40% higher in the light-treated retinas (n = 4, P < 0.001; Student’s t-test for all statistical analyses). The sums of the 24- and 22.5-kDa forms of the bFGF proteins were 102% higher in the light-treated retinas (n = 4, P < 0.001). (B) P83: Data for each band were averaged from lanes 1 through 3 (untreated control retinas) and lanes 4 through 6 (light-treated). The amount of the 18-kDa form of bFGF protein was 42% higher in the light-treated retinas (n = 3; P < 0.01). The sum of the 24- and 22.5-kDa forms of the bFGF proteins were 50% higher in the light-treated retinas (n = 3, P < 0.001).

bFGF Expression in Light-Treated P23H Retina

Because light stress in the RCS rat resulted in elevated bFGF expression that could be a cause of the observed photoreceptor rescue in this mutant, immunoblot analysis of bFGF was performed on illuminated P23H rat retinas to determine whether a similar response would be observed. P23H rats were treated with a single dose of 10 hours of bright light at P28. The rats were then returned to cyclic light, and retinas were collected 2.5 days or 5 days after treatment. Retinas of untreated littermates served as a control (Fig. 8). At 2.5 days after treatment, a 1.4-fold increase in the two high-molecular-weight forms (24 and 22.5 kDa) of bFGF protein was observed. A still larger 2.3-fold increase in bFGF protein was measured 5 days after treatment. Therefore, light treatment produced considerable upregulation of bFGF in the P23H retina.

DISCUSSION

Evidence collected in this study reveals a surprisingly long-lasting effect of the light-induced protective response in the RCS retina. A single exposure to intense light at P23 extended the survival of numerous photoreceptor nuclei to P60. By P83 the number of nuclei in light-treated retinas was still 3.5-fold higher than in untreated control retinas. The dose of light exposure used in the present work (120 fc for 10 hours) has been found to cause minimal damage to normal photoreceptors, together with maximal protection from subsequent light damage. Although it has been reported that the RCS rat is more sensitive to light damage than normal animals, it is apparent that the extent of damage caused by 10 hours’ exposure to 120 fc light at P23 is not significant and that the net outcome is a long-term increase in photoreceptor survival.

The increased survival of photoreceptors in the light-treated RCS retina was clearly reflected in enhanced retinal function as measured by the ERG. There was a large and significant difference in the ERG b-wave amplitudes of the light-treated RCS retinas and the untreated littermates. The b-wave amplitudes of the light-treated rats ranged from a factor of 1.7 to a factor of 11 times greater than those of the untreated rats. The barely detectable ERG observed in the present study in untreated RCS rats at P60 is in agreement with previous reports of reduced ERGs in RCS rats of similar age. The larger b-wave amplitudes in the light-treated rats are consistent with the presence of a larger number of functioning photoreceptors in the treated retinas. Previous studies in which b-wave amplitude was extensively used to measure retinal function in light damage and retinal degeneration models demonstrated that the b-wave am-
Amplitude is related to the number of remaining photoreceptors. A major increase in bFGF expression observed in light-stressed RCS retina is in agreement with previous studies in which light stress resulted in upregulation of bFGF in normal and RCS retinas. Evidence that bFGF is a trophic factor for photoreceptors comes from experiments in which intraocular injection of purified bFGF protected photoreceptors in inherited and induced retinal degeneration as well as in age-related photoreceptor degeneration. Adenovirally expressed bFGF was shown to protect photoreceptors in RCS rats. In the present study we were surprised to see that upregulation of bFGF protein after a brief exposure of RCS rats to intense light at P23 lasted for up to 2 months. That the long-lasting protection of photoreceptors is accompanied by an increase in bFGF protein may provide additional evidence that bFGF is involved in light-induced photoreceptor protection.

In the P23H rats, unlike the RCS rats, light stress did not result in photoreceptor rescue but in increased cell death. It has been proposed that exposure to intense light induces two opposing processes in the retina: a degenerating process that kills photoreceptors (light damage) and a protective response that enhances photoreceptor survival. Whereas light damage was minimal in the RCS rats after illumination for 10 hours at 120 fc, the same treatment caused significant photoreceptor loss in P23H rats. Additional experiments revealed that photoreceptors in the P23H rats were highly susceptible to light damage, because cell loss was also measured at much reduced light dosages. Thus, in the light-treated P23H rats, any possible protection would have been masked by the excessive light damage.

The increased susceptibility of the P23H rat to light damage over that seen in the RCS retina may be due to differences in the nature and site of the mutations. Although in the RCS rat photoreceptor degeneration is secondary to a receptor kinase mutation in the pigment epithelium, cell death in the opsin P23H rat is a consequence of a primary mutation in a photoreceptor-specific gene. The high susceptibility of the P23H rats to light damage is consistent with previous reports of similar findings in transgenic P23H mice. The abnormal dislocation of opsin, transducin, and phosphodiesterase to the inner segment and outer plexiform plasma membrane may increase photoreceptor vulnerability to insults. Susceptibility to light damage in opsin P23H mutants may also be a result of the prolonged life of phototransduction intermediates.

Because light treatment resulted in significant upregulation of bFGF in the P23H retina, it is apparent that the P23H retina is capable of responding to light stress in a manner similar to that seen in the RCS retina. It is noteworthy, however, that the
1.4-fold upregulation of bFGF in the P23H rat at 2.5 days after light treatment is lower than the 5-fold increase measured in RCS rats at 2.5 days after similar light treatment. Of note, a recent study reported that subretinal transplantation of normal neuroretinal cells prolongs survival of photoreceptors in RCS rats but not in P23H rats. It was proposed that higher levels of trophic factors may be necessary to promote rescue in the P23H retina. Thus, it is possible that light stress in the P23H rat does not produce sufficient levels of trophic factors to overcome intense death signals that are a result of both light damage and the mutation.

Our results do not rule out alternative explanations. For example, the expression and distribution of FGF receptors are known to be altered under pathologic conditions, which would certainly alter bFGF signaling in affected cells. Recently, FGF soluble receptor (SR1) was found to inhibit bFGF activity during retinal degeneration. An increase of SR1 expression could also counterbalance the bFGF increase.

In conclusion, the use of light to recruit endogenous survival factors may circumvent potential harmful side effects, such as cataracts and angiogenesis, which were observed in RCS retinas after treatment with exogenous bFGF. However, in view of the negative results that were obtained with the light-treated P23H rats, it is necessary that the extent of sensitivity to light damage and levels of upregulation of trophic responses first be determined. Mutations with low sensitivity to light damage can then be considered for noninvasive light therapy.

### References


