Light Treatment Enhances Photoreceptor Survival in Dystrophic Retinas of Royal College of Surgeons Rats

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Purpose. To determine whether treatment with bright light elicits a protective response that enhances photoreceptor survival in Royal College of Surgeons (RCS) rats with inherited retinal degeneration.

Methods. RCS rats were illuminated for 10 to 12 hours with 130 foot-candles (fc) of white or green light. Untreated littermates that were kept under low cyclic light levels were used as control subjects. Photoreceptor survival was determined by quantitative analysis of photoreceptor nuclei and ultrastructural assessment of cellular organization. Basic fibroblast growth factor (bFGF) and ciliary neurotrophic factor (CNTF) gene expression were determined at the mRNA and protein levels.

Results. Treatments of RCS rats with a single dose of bright light on postnatal day 23 (P23) greatly enhanced photoreceptor survival. Ultrasturctural analysis revealed intact inner segments in light-treated retinas, whereas in untreated retinas only remnants of inner segments were observed. By P42, numerous viable nuclei were counted in the posterior retina of light-treated rats, whereas most of the remaining nuclei in untreated RCS rat retinas were highly pyknotic. At 2.5 days after treatment with a single dose of bright light, bFGF gene expression was significantly higher than in untreated RCS rat retinas. By P42, bFGF protein levels were still significantly higher in the treated retinas.

Conclusions. Exogenous bFGF has been shown to promote photoreceptor survival in the RCS rat retina. Thus, the increased bFGF expression that was measured in the light-treated RCS rat retinas may be a protective response to light stress, which supports the observed rescue of photoreceptors in light-treated RCS rat retinas. (*Invest Ophthalmol Vis Sci.* 1999;40:2383–2390)

In RCS rats with inherited retinal dystrophy, a mutation expressed in the pigment epithelial (PE) cells affects the phagocytosis of shed rod outer segment (ROS) membranes. The altered PE function leads to accumulation of membranous debris in the subretinal space and to photoreceptor cell death.[1]

A successful therapeutic attempt to enhance photoreceptor survival in the RCS rat retina was achieved by intravitreal injection of exogenous basic fibroblast growth factor (bFGF).[2] In other studies, endogenous levels of bFGF and CNTF were shown to increase in response to focal mechanical injury to rat retinas.[3] Elevation in growth factors as a protective response to injury may underlie the enhanced survival of photoreceptors that was observed in dystrophic RCS rat retinas[4] and in light-damaged normal rat retinas.[3,5]

Nonintrusive environmental insults such as light stress also increase bFGF gene expression in rat retinas.[6] The bFGF upregulation during light stress may function as the endogenous survival–rescue factor that prevents photoreceptor degeneration when animals are subsequently exposed to damaging light conditions.[6] This possibility was confirmed in a recent study which demonstrated that a short preconditioning period of normal rat retina with bright light resulted in upregulation of bFGF and CNTF and protection against photoreceptor loss during a follow-up period of extended exposure to damaging light.[7]

In the present study we explored whether light stress can be used to elicit a protective response that enhances photoreceptor survival in the retina of dystrophic RCS rats. Light treatments were administered between the second and third postnatal weeks when most of the photoreceptors are still viable,[8] and metabolic capabilities such as opsin synthesis are maintained at relatively high levels.[9] The data obtained in this study revealed a major elevation in bFGF gene expression and significant enhancement of photoreceptor survival in the light-treated RCS rat retina.

Materials and Methods

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

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Animals

Tan-hooded, pink-eyed RCS rats were raised in a 12-hour dark–12-hour light cycle at low (5 foot-candles [fc]) light levels. Normal Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were kept in the same illumination conditions for at least 2 weeks before experimentation.

Bright Light Treatments

Bright white light of 115 to 130 fc was produced by two fluorescent lamps, 40 W each (GE, cool light) at a distance of 60 cm above the cage floor.[10] After a preconditioning protocol with bright light,[7] rats were exposed to a single dose of 10 to 12 hours' bright light at P18, P23, or P30. In some experiments rats were exposed to two dosages of bright light, at P18 and P23 or at P23 and P30. Exposure to bright light was performed during the light period of the diurnal cycle. Control untreated littermates were kept in low cyclic light levels (5 fc) and killed together with the treated rats. In some experiments the rats were illuminated with green light. A halogen lamp (300 W) was placed above the cage, and the light was filtered through a green sheet of plexiglas (no. 2092). The peak of the transmitted light was measured at 520 nm. Light intensity at the cage floor was 130 fc. Temperature inside the cage was maintained at 22°C to 24°C.

Microscopy

Rats were killed by an overdose of CO_2, and eyes were quickly enucleated and placed in a fixative (4% formaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer). After 30 minutes in fixative, the eyes were bisected along the vertical meridian. The two hemispheres were fixed for 3 hours. The tissue was then treated with 1% OsO_4, embedded in araldite, and viewed by light and electron microscopes.

Measurement of Photoreceptor Layers

Light microscopy was used for quantitative assessments of photoreceptor survival.[8] Sections 1-μm thick were cut along the vertical meridian of light-treated and untreated retinas. The sections include the full length of the retina from the optic nerve head to the ora serrata.[8] The width of photoreceptor outer nuclear layer (ONL) was measured at 100- to 150-μm intervals along the posterior periphery axis using an eyepiece graticule. Measurements were obtained in the superior and inferior hemispheres. Thin sections that were cut from the same regions of the retina were used for ultrastructural analysis.

RNA Preparation and Northern Blot Analysis

Retinas were dissected, snap frozen in liquid nitrogen, and stored at −80°C. Total RNA was obtained using a kit (Rneasy Total RNA assay; Qiagen, Chatsworth, CA) according to the manufacturer's instructions. Northern blot analysis was performed as previously described.[3] Briefly, total RNA (15 μg per sample) was electrophoresed on agarose gels and transferred to a nylon membrane. Random primed ^{32}P-labeled cDNA probes for rat bFGF, CNTF, or 18s rRNA (10^6 cpm/ml) were added to the hybridization buffer and hybridized at 50°C overnight. Blots were exposed to a storage phosphor screen, and data were digitized by scanning the phosphor screen with a phosphor imager (Phosphor Imager System; Molecular Dynamics, Sunnyvale, CA). Quantitative analysis of the digitized data was performed using image analysis software (Image Quant; Molecular Dynamics).

Protein Preparation and Immunoblot Analysis

Protein preparation and immunoblot were performed as previously described.[7] Total protein was obtained by homogenizing retinas in a lysis buffer. The tissue homogenate was centrifuged, supernatant collected, and the protein concentration determined. Total proteins (100 μg) of each sample were size fractionated on sodium dodecyl sulfate (SDS)–polyacrylamide gels. Blots were probed with anti-bFGF or anti-CNTF antibodies. Signals were recorded on film and scanned on a densitometer. Quantitative analysis of the digitized data was performed using the same image analysis software.

Results

Cytologic Observations

A major increase in photoreceptor survival, determined by the ONL width at P42, was achieved in RCS rats that were illuminated with a single dose of bright light at P23. Illumination of younger rats at P18 did not produce a measurable increase in photoreceptor survival, indicating that the mechanism underlying the rescue response is not yet developed. Illumination of rats at P30 resulted in a rescue that was lower than that seen in rats treated at P23. The reduced survival in P30-treated rats was apparently because of a significant level of cell death at that age, evidenced by the increased number of pyknotic nuclei by P30.[8] Additional experiments revealed that two doses of illumination, one at P23 and the second either earlier (at P18) or later (at P30) also did not improve the survival over that seen with a single dose at P23. Therefore, a single dose of bright light at P23 was sufficient to induce optimal enhancement of photoreceptor survival in the RCS rat.

The most pronounced increase in photoreceptor survival was seen in the posterior retina, in a region extending from the optic nerve head past the equator, a distance of 0.1 to 3 mm from the optic nerve head. Figure 1 depicts the photoreceptor layers in the posterior retina of light-treated and untreated RCS rats at P42. In the light-treated retina the ONL layer had areas of up to six rows of nuclei (Fig. 1A). The same region in untreated RCS rats had approximately three rows of nuclei (Fig. 1B). At the ultrastructural level, most of the surviving nuclei in light-treated retinas had a characteristic morphology of normal nuclei with dispersed chromatin (Figs. 2 and 5), whereas in untreated retinas many nuclei were condensed and highly pyknotic (Fig. 3), an indication of ongoing loss of viability. In the retinal periphery the difference in ONL width between the treated and untreated retinas was less pronounced, although many of the remaining nuclei in the periphery of untreated retinas were also highly pyknotic (Fig. 3).

A significant characteristic of the light-treated retinas was the presence of a distinct, clearly defined rod inner segment (RIS) layer along the posterior periphery axis (Fig. 1A). Ultrastructural analysis revealed the presence of well-preserved inner segments both in the posterior (Figs. 4 and 5) and the periphery (Fig. 2) of light-treated retinas. In untreated retinas the RIS layer in the posterior retina almost completely disappeared by P42 (Fig. 1B). In the periphery of untreated retinas the remaining RIS layer consisted of degenerating remnants (Fig. 3).

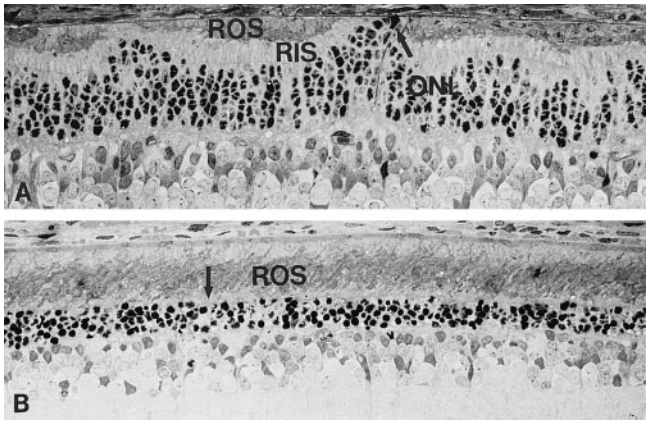

FIGURE 1. Posterior retinas in light-treated and control untreated RCS rats at P42. (**A**) Rat treated with bright light (130 fc) at P23. Large numbers of photoreceptor nuclei are seen in the ONL. Up to six rows of nuclei are present. A clearly defined RIS layer is observed. The ROS is of uneven width. In certain areas the ROS layer is almost completely absent. At some points devoid of RIS and ROS, the ONL extended to Bruch's membrane (*arrow*). (**B**) Untreated rat retina. A major reduction in width of the photoreceptor nuclear layer is observed. Remaining nuclei are highly condensed (pyknotic). The RIS layer (*arrow*) is greatly reduced. The ROS debris layer is wide.

Thinning of the ROS debris layer was observed in the light-treated retinas. It was most pronounced in the posterior to the midperiphery, in both the superior and inferior hemispheres. At several sites in the posterior region of light-treated retinas photoreceptor nuclei penetrated through the outer limiting membrane and the inner and outer segment layers and reached Bruch's membrane (Fig. 1A). Damage to the PE layer was observed at points where the ONL reached Bruch's membrane and at some other sites along the posterior retina (Fig. 4). In other areas the PE layer appeared to be intact (Fig. 5).

Exposure of RCS rats to 12 hours of 130 fc of green light at P23 extended photoreceptor survival as was observed with white light of the same intensity and duration. The green light produced similar alterations in photoreceptors and PE cells as that seen with white light (data not shown).

Illumination of normal Sprague–Dawley rats with a single dose of 12 hours' bright white light resulted in limited photoreceptor damage in the posterior retina of the superior hemisphere. Electron microscopic analysis of these retinas did not reveal the damage to PE cells that was seen in light-treated RCS rats (data not shown).

Quantitative Analysis of Photoreceptor Layers

The extent of photoreceptor survival was determined by measurements of the ONL layer. Data of ONL width in the posterior-to-equatorial retina, a distance of 0.1 to 2 mm from the optic nerve head in the superior and inferior hemispheres, are presented in Figure 6. A group of treated RCS rat retinas were compared with untreated RCS rat retinas. The data clearly demonstrate the increased survival of photoreceptors in treated retinas. The average ONL width of all the treated retinas was 18.2 ± 0.53 μm (n = 9), whereas in untreated retinas the average ONL width was 11.9 ± 0.36 μm (n = 5), a difference of approximately 35%. Furthermore, many of the remaining nuclei in the untreated rats that contribute to the ONL width were highly pyknotic at P42. It is noteworthy that although there was no significant difference in ONL width between the superior and inferior hemispheres in untreated retinas, in several treated retinas the ONL width in the inferior retinas was more than the width of ONL in the superior retina (Fig. 6).

To determine the relationship between photoreceptor survival and changes in ROS debris layer, the widths of ONL, RIS, and ROS layers were compared. The data were collected from P42 RCS rat retinas that were treated with a single dose of light at P23 and from untreated RCS rat retinas at P42. In untreated retinas the ROS debris layer formed a wide band of a largely constant width (approximately 25–30 μm) between the posterior and periphery. A narrow band of inner segment remnants was measured only in the periphery (data not

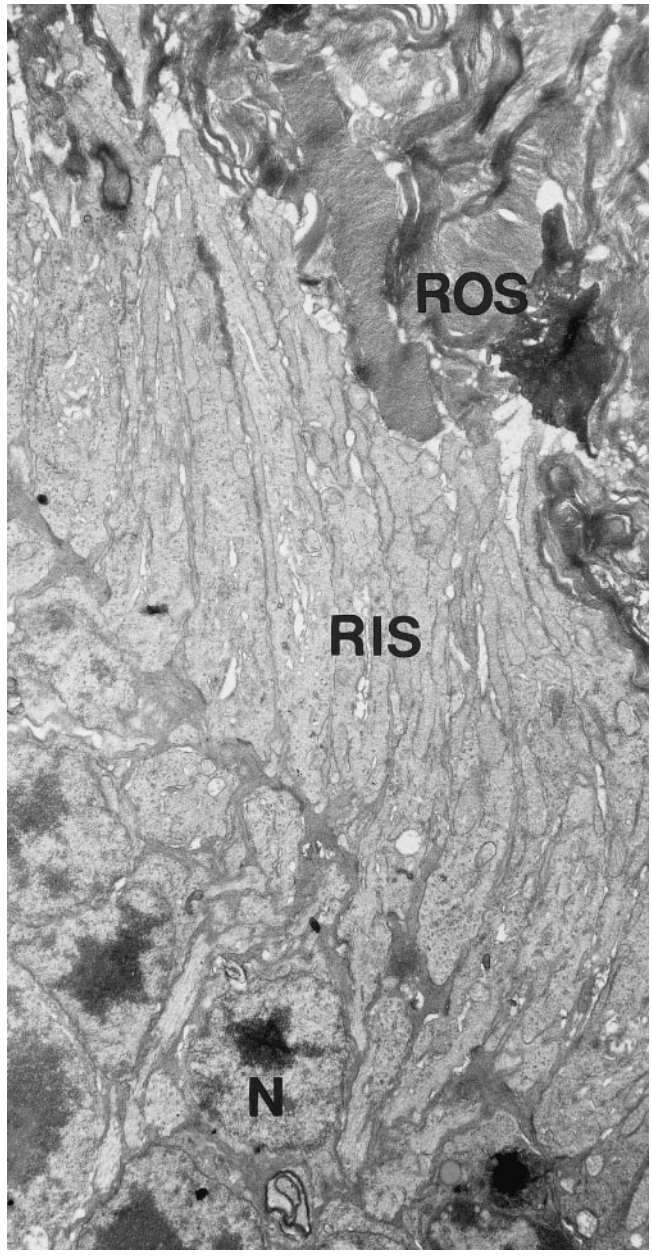

FIGURE 2. Light-treated RCS rat at P42. Peripheral retina. Normal morphology of nuclei (N) is characterized by dispersed chromatin. Well-preserved RISs are present. ROS debris layer typical in the RCS rat retina is seen. Magnification, ×9800

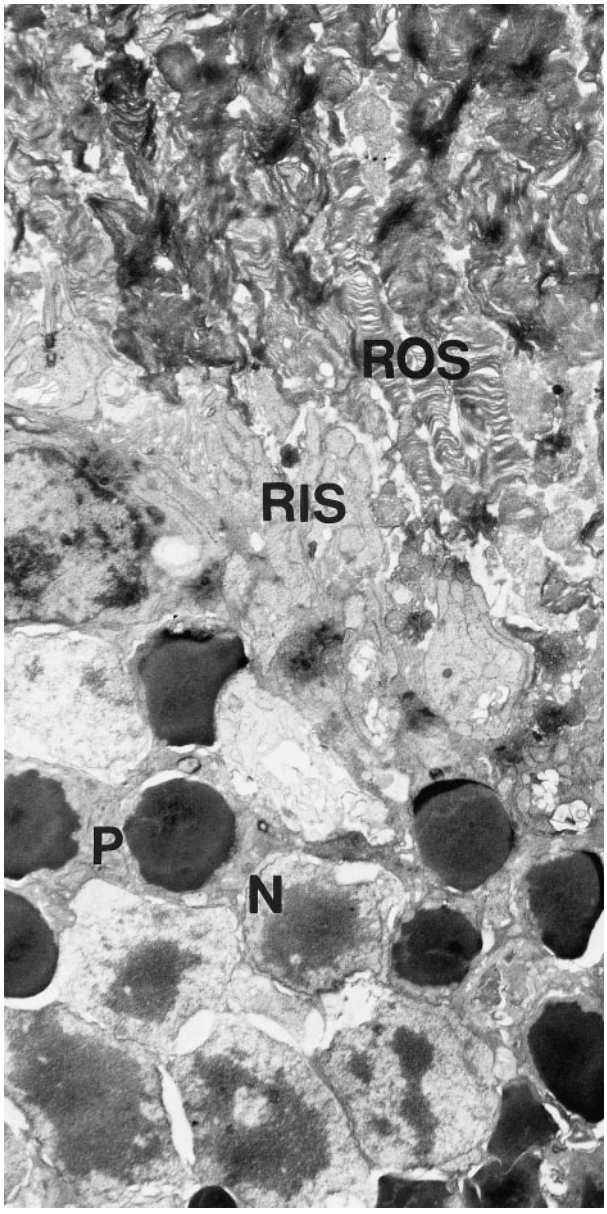

FIGURE 3. Peripheral retina of untreated RCS rat at P42. A number of nuclei with condensed (pyknotic) chromatin (P) are seen. Some of the nuclei in this region have normal morphology (N). The RIS layer consists of small degenerated structures. Magnification, ×4900.

shown). In treated rats the width of the ROS layer varied greatly along the posterior periphery axis. A typical width distribution of the various layers in a treated retina is presented in Figure 7. Note that a wide ONL layer was measured in areas with wide or narrow ROS debris layer (Fig. 7). In addition to variations in width, substantial reduction in electron density of the membranous debris and vesiculation of the ROS discs were observed in the posterior retina (Fig. 4). Such alterations were not observed in the periphery (Fig. 2).

Expression of bFGF and CNTF

Initial experiments were designed to determine whether light treatments cause upregulation of bFGF and CNTF shortly after light exposure, as previously shown in normal rat retinas.[7] Thus, levels of bFGF and CNTF gene expressions were determined in isolated retinas 2.5 days after treatment of RCS rats with a single dose of 12 hours' bright light at P23. Untreated littermates of the same age were used as control animals. A significant increase in bFGF mRNA, 2.5 times the control level, was observed after light treatment (Fig. 8A). bFGF protein analysis showed a fivefold increase in bFGF levels after light treatment in the two high-molecular forms of 24-kDa and 22.5-kDa, whereas there was no change in the 18-kDa bFGF protein (Fig. 8B). No significant alterations in either mRNA or protein levels of CNTF were observed (data not shown).

In view of the substantial level of photoreceptor survival that was observed in light-treated RCS rat retinas at P42, expression of bFGF protein was analyzed in retinas at P42 after a single dose of 10 hours of bright light at P23. Untreated littermates of the same age were used as control animals. The results presented in Figure 9 show significantly higher levels of bFGF at the three molecular weights of bFGF. There was an average increase of 9.5-fold in the 24-kDa and 22.5-kDa proteins, and a 4.5-fold increase in the 18-kDa bFGF protein. Therefore, a single light treatment at P23 was sufficient to cause a prolonged elevation of bFGF expression in the RCS rat retina that persisted for at least 19 days.

The correlation between upregulation of bFGF and photoreceptor rescue was further evaluated by analysis of bFGF

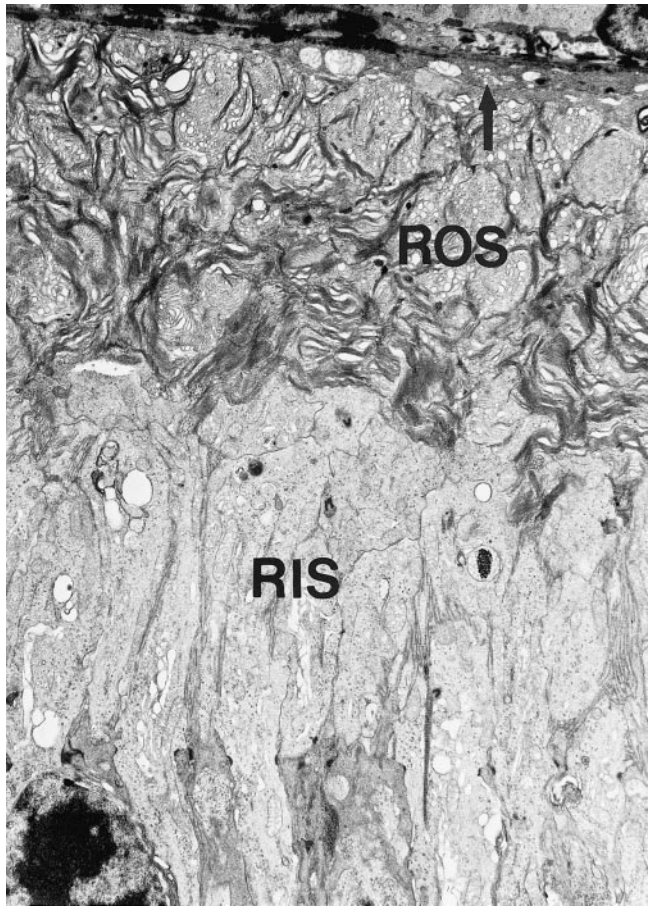

FIGURE 4. Posterior retina and light-treated RCS rat at P42. Pigment epithelium cell layer (*arrow*) is damaged or absent. Vesiculation of the photoreceptor disc membranes in the debris layer (ROS) is seen. Magnification, ×5000.

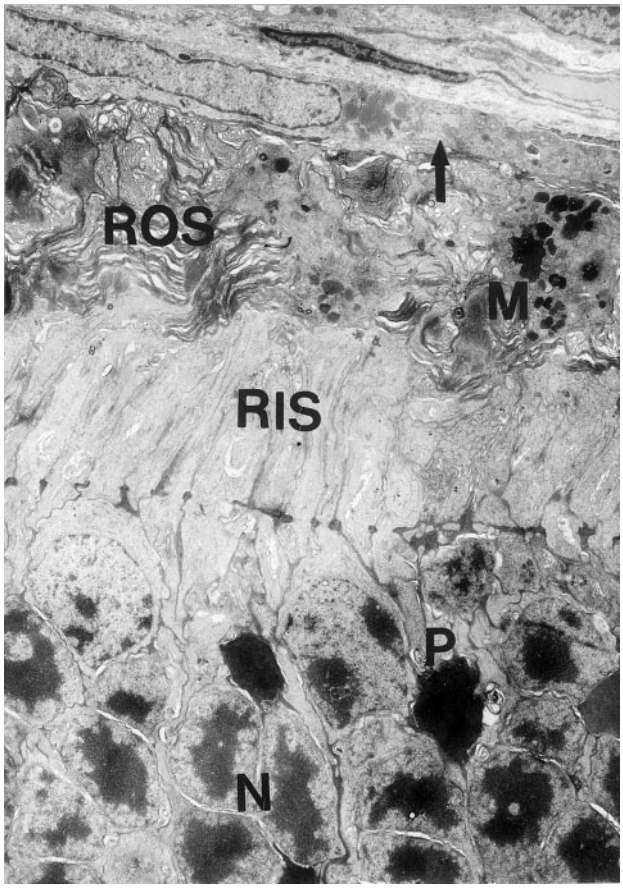

FIGURE 5. Equatorial retina of the light-treated RCS rat at P42. Pigment epithelial cells (*arrow*) appears to be intact. The RISs are well preserved. The ROS debris layer is reduced in size. A macrophage (M) could be seen in the ROS debris layer. Most of the photoreceptor nuclei show normal morphology (N), and only a few are pyknotic (P). Magnification, ×3000.

protein upregulation in P18 rats in which light treatment did not result in a measurable increase in photoreceptor survival. In this experiment, RCS rats were treated with a single dose of 10 hours' bright light at P18. The rats were then analyzed for bFGF levels 2.5 days after illumination and compared with nontreated littermates. A third group of littermates were illuminated at P23 and also analyzed for bFGF levels 2.5 days after illumination. The results presented in Figure 10 show a very small (1.2-fold) upregulation of bFGF in rats treated at P18 whereas illumination at P23 induced a major increase (5.6-fold) in bFGF levels.

DISCUSSION

Preconditioning of neurons to survive damaging insults by delivering a sublethal insult of the same type was demonstrated in various studies. It was suggested that the limited insult is sufficient to elicit an endogenous protective response that enables the cells to sustain a damaging insult of a higher magnitude.[11] In light-damage experiments, rats exposed to bright light for a limited time were less sensitive to subsequent damage by prolonged light exposure.[7]

Localized protective response in the RCS rat retina was previously demonstrated with mechanical damage[2,4] and laser burns,[12,13] both of which elicit limited enhancement of photoreceptor survival at the site of injury. In the present study we used light stress to induce a protective response in the RCS rat retina. This approach resulted in a global effect that encompassed large parts of the retina. A single dose of 10 to 12 hours of bright light at P23 was sufficient to extend photoreceptor survival beyond P42. A large number of viable photoreceptor nuclei were present in light-treated retinas, whereas mostly pyknotic nuclei remained in untreated RCS rat retinas of the same age.

The mechanism by which bright light enhances photoreceptor survival may be associated with increased availability of growth factors. Upregulation of bFGF by light was previously shown in normal rats that were maintained for 3 weeks under a diurnal cycle with light levels of 55 to 70 fc. In situ hybridization of bFGF mRNA localized an intense signal over the inner segments.[6] It was suggested that light stress increases synthesis of bFGF and that this is one of the endogenous rescue molecules that promote photoreceptor survival when challenged with constant light.[6] Treatment of normal rats with higher light intensities for shorter times, which protects

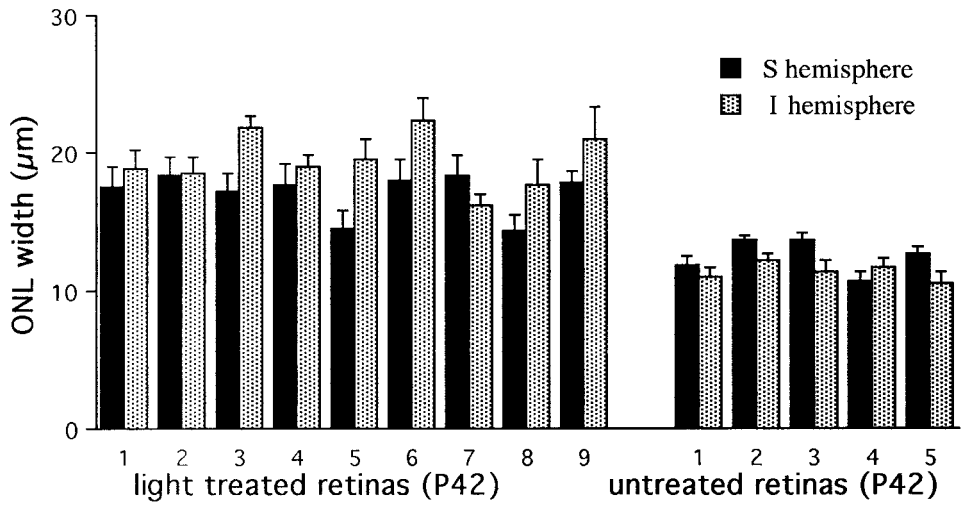

FIGURE 6. Quantitation of ONL width as a measure of photoreceptor survival. Retinas were from P42 RCS rats treated for 12 hours with bright light at P23 (*left*) and untreated retinas (*right*). Each column depicts one retina with a superior hemisphere (*solid bar*) and an inferior hemisphere (*dotted bars*). Measurements were made in the posterior retina along the vertical meridian, in an area that extends 0.1 to 2 mm from the optic nerve head toward the equator. A total of 13 to 16 measurements 100 to 150 μm apart were made at each hemisphere (n = 13–15).

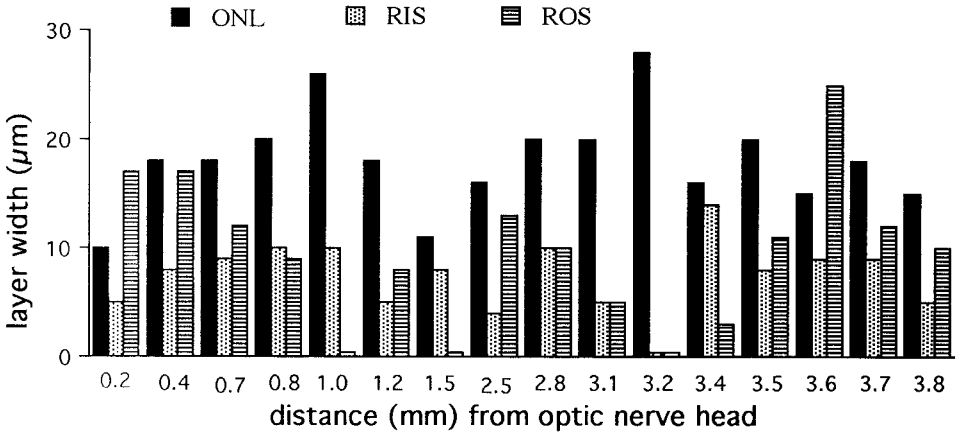

FIGURE 7. Quantitation of the width of photoreceptor nuclei (ONL), RIS, and ROS layers. A representative retina from a P42 RCS rat treated for 12 hours with bright light at P23 is shown. Measurements were made at 16 sites along the full length of one hemisphere from the posterior (*left*) to the peripheral (*right*) retina. At each point, the widths of ONL, RIS, and ROS are depicted. The data at 3.2 mm are for ONL width only. This is the point at which the ONL reached Bruch's membrane, and it is devoid of ROS and RIS (see Fig. 1A).

against subsequent constant light damage also results in elevation of bFGF gene expression.[7] In situ hybridization experiments revealed the presence of bFGF mRNA in the PE cell layer, the inner nuclear layer, and the photoreceptor inner segments.[14] Müller cells might be an additional source of bFGF in light-stressed retinas. Studies of cultured rat Müller cells showed that the cells respond to bFGF by elevation of bFGF gene expression.[15] Thus, release of bFGF from an endogenous reservoir in case of injury could lead to the production of bFGF by Müller cells, which in turn may enhance photoreceptor survival.[15] A direct role for bFGF in promoting photoreceptor viability was suggested by various studies. Transgenic mice carrying mutant bFGF receptors undergo progressive retinal degeneration.[16] Recent studies have shown that bFGF directly stimulates the survival of mature mammalian photoreceptors in culture.[17] In light-damage experiments, a role for bFGF in preventing nitric oxide toxicity has been suggested.[18]

In view of the significant rescue of photoreceptors in RCS rats that were treated at P23, bFGF levels were measured after light treatment at that age. Analysis of bFGF expression revealed a major elevation of both mRNA and protein levels at 2.5 days after treatment with a single dose of bright light. This observation is in agreement with the kinetics of bFGF upregulation that was measured in light-treated normal rat retinas,[7] although similar upregulation of CNTF was not seen in the light-treated RCS rat retinas. It is possible that the absence of CNTF upregulation was caused by species differences. The effect of a single light treatment was long lasting, because a major increase in bFGF protein level was measured 19 days after treatment. Furthermore, the upregulation of bFGF in light-treated retinas may extend for a longer period, because preliminary experiments revealed considerable photoreceptor survival at P60 in treated RCS rats. The correlation between upregulation of bFGF and photoreceptor survival in light-treated rats was further enhanced by the observation that illumination at P18, which did not increase photoreceptor survival, also did not increase bFGF expression. It is possible that at P18 the machinery responsible for upregulation of bFGF in response to light stress is not yet in place.

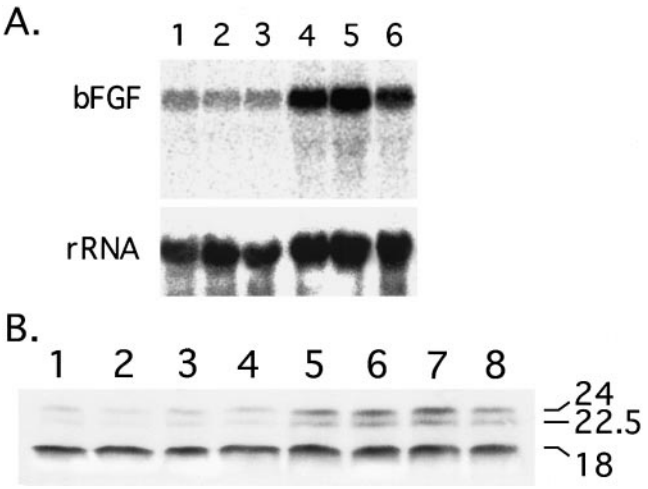

FIGURE 8. Expression of bFGF mRNA and proteins 2.5 days after light treatment for 12 hours with bright light at P23. (**A**) Expression of bFGF mRNA was assessed by northern blot analysis. Data from six RCS rats, three untreated control animals, and three treated littermates are presented. Each lane shown in (**A**) represents a sample of 15 μg total RNA from two retinas (*lane 1* through *3,* control animals; *lane 4* through *6,* 2.5 days after light treatment). A major bFGF transcript of 7.0 kb was detected in all lanes (*top*). The blot was stripped and rehybridized with probes for 18s rRNA, which served as a control for RNA loading (*bottom*). Signals of the 7.0-kDa bands were averaged from *lanes 1* through *3* (control) and *4* through *6* (treated) and normalized with averaged signals of the 18s rRNA. (**B**) The amount of bFGF proteins was determined by immunoblot analysis. Data from eight RCS rats, four untreated control animals and four treated littermates are presented. Each lane shown in B represents a sample of 100 μg total protein from two retinas (*lane 1* through *4,* control animals; *lane 5* through *8,* 2.5 days after light treatment). bFGF proteins were detected as three distinct bands of 24, 22.5, and 18 kDa.

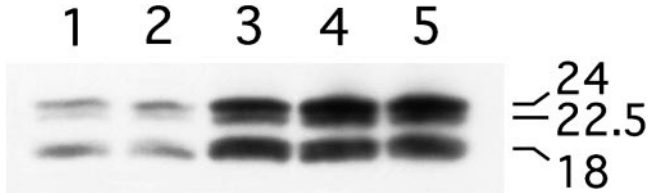

FIGURE 9. Expression of bFGF proteins at P42, 19 days after a single treatment with bright light for 10 hours at P23. The amount of bFGF proteins was assessed by immunoblot analysis. Data from five RCS rats, two untreated control animals and three treated littermates are presented. Each lane represents a sample of 100 μg total protein from two retinas (*lanes 1* and *2,* control animals; *lanes 3* through *5,* light treatment). Proteins of bFGF were detected as three distinct bands of 24, 22.5, and 18 kDa.

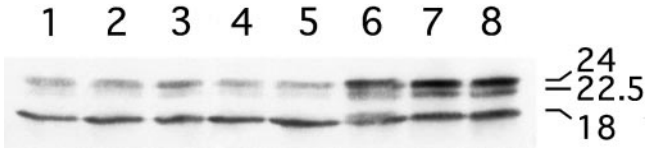

FIGURE 10. Immunoblot analysis of bFGF levels in RCS rats treated with a single dose of 10 hours' bright light at P18 versus P23. Data from eight RCS rats of the same litter: illuminated at P18 (*lanes 1* through *3*), nontreated control animals (*lanes 4* through *5*), illuminated at P23 (*lanes 6* through *8*). Each lane represents a sample of 100 μg total protein from two retinas. The retinas were isolated 2.5 days after treatment. Significant differences in levels of high molecular forms of bFGF are seen. Ratio of bFGF 22.5-kDa and 24-kDa bands in rats treated at P18 (*lanes 1* through *3*) versus control animals (*lanes 4* and *5*) is 1.22. Ratio of the same bands in rats treated at P23 (*lanes 5* through *8*) versus control rats is 5.67.

Analysis of endogenous bFGF expression in RCS rat retinas revealed lower levels of bFGF at P21 compared with normal retinas of the same age,[19] although similar reduction in bFGF have not been found in dystrophic mice retinas.[6] Other studies have demonstrated that a single injection of bFGF is sufficient to promote photoreceptor survival in RCS rats for at least 2 months.[2] Therefore, increased levels of bFGF in the RCS rat retina either by exogenous application or by upregulation of endogenous levels by light treatment, as shown in the present study, may provide needed neurotrophic support to photoreceptors in the bFGF-deficient retina.

Additional alterations in the retina that could be considered as factors in enhancing photoreceptor viability are changes in the ROS debris layer in light-treated RCS rat retinas. The accumulated ROS membranous debris in the subretinal space may hinder the diffusion of oxygen and nutrients from the choroid, which could affect photoreceptor viability. Thus, the thinning of the ROS debris layer in light-treated rats may enhance viability by reducing a diffusion barrier. However, as shown in Figure 7, there was no inverse correlation between the width of the ROS debris layer and ONL layers. Furthermore, dual light treatments at P18 and P23 or at P23 and P30, which further reduced the ROS debris layer, did not increase the survival of photoreceptors. In other studies with RCS rats, reduction in the debris zone by macrophage transplants had little effect on photoreceptor cell survival.[20] Therefore, thinning of the ROS debris layer induced by bright light was probably not a significant factor in enhancing photoreceptor survival in treated RCS rat retinas.

Damage to PE cells was observed in the posterior retina of light-treated RCS rats. At some points in the posterior retina with damaged PE cells, photoreceptor nuclei were found next to Bruch's membrane. Similar relocation of photoreceptor nuclei has been described in type I light damage, which involves damage to PE cells.[21,22] The damage to PE cells seen in light-stressed RCS rat retinas was not observed in normal Sprague–Dawley rat retinas that were exposed to an identical treatment of bright light. Although the mechanism of retinal light damage is not yet understood, recent studies confirm the oxidative nature of the process.[23] It is possible that the increased susceptibility of PE cells in the RCS rat is caused by exposure to oxidative mediators released from altered ROS debris layer in light-treated retinas.

Differences in the level of photoreceptor rescue in the superior and inferior retinas were observed in the treated RCS rats, with better survival generally measured in the inferior retina. Because the rate of photoreceptor degeneration in pink-eyed RCS rats is the same in the superior and inferior hemispheres,[8] it can be assumed that differences in rates of photoreceptor survival are caused by different levels of exposure to the bright light. Analysis of normal Sprague–Dawley rats exposed to the same level and duration of bright light revealed localized damage in the posterior region of the superior hemisphere. This observation is in accordance with the known susceptibility of the posterior region in the superior retina to light damage.[24] Thus, the free-roaming RCS rats that were exposed to more than optimal levels of light during the 10- to 12-hour illumination period may have had some cell loss due to light damage in the superior hemisphere, in addition to enhancement of cell survival.

Treatments of RCS rats with bright green light produced the same qualitative results as seen with the white light, including type I light damage to PE cells. Therefore, the involvement of rhodopsin in mediating the observed changes in photoreceptors and PE cells can be assumed. The observed type I light damage to PE with green and white light is in agreement with data that show similar damage to photoreceptor and PE by green and short-wavelength light.[25] In view of the damage caused by light to the PE cells, adverse effects of light treatment should be considered. In further studies, fine calibration of light brightness and duration of exposure will be required to obtain optimal conditions that will be adequate to elicit a response to light stress without causing PE cell damage. The possibility of prolonging photoreceptor survival by noninvasive treatment such as sublethal levels of light is an interesting mode of therapy. Complications of exogenous applications of bFGF such as development of cataracts in bFGF therapy[26] and other potentially harmful side effects may be avoided. The potential of light treatment as a therapeutic mode will be explored in other models of retinal degeneration.

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