The Melatonin Antagonist Luzindole Protects Retinal Photoreceptors from Light Damage in the Rat

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PURPOSE. Systemic administration of melatonin can increase retinal light damage in the rat. The role of retinal melatonin receptors in modulating light-damage susceptibility was investigated by intravitreally injecting the melatonin receptor antagonist luzindole into rats.

METHODS. Nine Sprague-Dawley albino rats 8 to 9 weeks of age were kept in 50 lux cyclic light for at least 7 days before receiving an intravitreal injection of 1 μl 1 mM luzindole in one eye and 1 μl vehicle in the other eye. The injection was given just before the beginning of the normal 12-hour dark phase. At the end of this dark period, animals were exposed to constant light of 2500 lux for 48 hours. Animals were returned to dim cyclic light for 7 days, and dark-adapted electroretinograms (ERGs) were then recorded from the two eyes simultaneously. The eyes were processed for retinal morphology. Photoreceptor nuclei were counted in the outer nuclear layer (ONL), and the thickness of the ONL and that of the rod outer-segment plus inner-segment layer were measured at several points along sections through the vertical meridian. Two age-matched control rats were maintained in dim cyclic light but received no injections.

RESULTS. Luzindole-treated eyes had ERG b-wave thresholds of 2.7 ± 0.5 (mean ± SEM) log candela (cd)/m² lower than the fellow eyes injected with vehicle (P < 0.001), and the maximum b-wave amplitude was 1.0 ± 0.2 log μV greater in luzindole-treated eyes (P < 0.001). Thresholds of the scotopic threshold response were 0.5 ± 0.1 log cd/m² lower than those in vehicle-injected eyes (P < 0.05). Luzindole-treated eyes on average had twice as many photoreceptor cells remaining (P < 0.005). In some areas, several rows of photoreceptor nuclei and outer segments remained in the luzindole-treated eye, whereas the fellow control eye showed cells only occasionally and no outer segments.

CONCLUSIONS. Eyes pretreated with the melatonin receptor competitive antagonist luzindole before the dark phase preceding constant light exposure were substantially protected from light damage to the retinal photoreceptors. These results implicate the intraocular melatonin-dopamine system in the regulation of light-damage susceptibility. (Invest Ophthalmol Vis Sci. 1998;39:2458-2465)

Retinal light damage is a model for studying photoreceptor degeneration. Low-to-moderate levels of illumination reliably produce easily quantified photoreceptor cell death in the rat in a relatively short time (see Ref. 1 for review), which makes this model attractive for testing potential drug treatments that may preserve photoreceptor structure and function in disease.

We have been studying endogenous retinal neuromodulators that are involved in photoreceptor cell metabolism and that may modulate light-damage susceptibility (see Refs. 2 and 3 for review). Melatonin (N-acetyl-5-methoxytryptamine) is a retinal neuromodulator that is synthesized in the pineal gland and the retina at low rates during daylight hours and at higher rates at night. In the retina, melatonin plays a key role in rhythmic photoreceptor metabolism and in regulation of the release of dopamine from post-photoreceptor neurons. Melatonin and dopamine have mutually antagonistic effects on retinal physiology and on their own synthesis and release. Light-damage susceptibility is enhanced under conditions that would be expected to elevate retinal melatonin levels, such as during the normal dark period or after previous exposure to darkness. However, when retinal melatonin levels are low, such as during mid-daylight hours or immediately after a period of light adaptation, damage from bright light exposure is reduced. Conversely, melatonin injected systemically enhances susceptibility to light-induced photoreceptor damage in rats. However, it is unknown whether systemic melatonin acted directly on receptors in the retina or indirectly by affecting other circulating factors.

Luzindole is a competitive melatonin receptor antagonist that has been shown to inhibit the ability of melatonin and darkness to reduce the release of dopamine from retinal neurons. To investigate whether local retinal melatonin receptors modulate light-damage susceptibility, we used intravitreal injections of luzindole to block these receptors in the rat in vivo just before the pre-exposure dark period. We found that this resulted in substantial protection from light damage, indicating...
TABLE 1. Effects of Unilateral Intravitreal Injection of Luzindole before Light Exposure on the ERG and Photoreceptor Histology in Light-damaged Rats

<table>
<thead>
<tr>
<th>Animals</th>
<th>ONL Thickness (µm)</th>
<th>ONL Cell Count</th>
<th>ROS+IS Length (µm)</th>
<th>B-Wave Threshold (log cd/m²)</th>
<th>B-Wave V max (log µV)</th>
<th>STR Threshold (log cd/m²)</th>
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<tr>
<td>Rat 5</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>R</td>
<td>24.7</td>
<td>1568</td>
<td>22.7</td>
<td>-2.8</td>
<td>2.5</td>
<td>-4.1</td>
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<tr>
<td>L</td>
<td>14.7</td>
<td>1182</td>
<td>13.2</td>
<td>-1.8</td>
<td>2.0</td>
<td>-3.8</td>
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<td>9.5</td>
<td>1.0</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Rat 6</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>R</td>
<td>10.3</td>
<td>759</td>
<td>5.3</td>
<td>-2.1</td>
<td>1.9</td>
<td>-4.5</td>
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<tr>
<td>L</td>
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<td>455</td>
<td>2.1</td>
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<td>R</td>
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<td>3.0</td>
<td>0.8</td>
<td>0.30</td>
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</table>

Average

| R       | 14.4 ± 2.6        | 899 ± 170      | 9.4 ± 3.4          | -1.6 ± 0.4                  | 1.9 ± 0.2             | -4.2 ± 0.1               |
| L       | 7.2 ± 2.1         | 466 ± 188      | 3.2 ± 2.5          | 0.9 ± 0.8                   | 1.0 ± 0.3             | -3.8 ± 0.3               |
| Dif.    | 7.1 ± 1.4         | 433 ± 43       | 6.3 ± 1.2          | 2.5 ± 0.6                   | 0.9 ± 0.3             | 0.3 ± 0.1                |
| P       | 0.01              | 0.001          | 0.05               | 0.05                        | 0.05                  | 0.05                     |

Undamaged no-treatment control (n = 4)

| R       | 35.5 ± 4.7        | 3423 ± 54      | 39.0 ± 0.5         | -3.8 ± 0.2                  | 3.2 ± 0.1             | -7.2 ± 0.4               |
| L       | 36.4 ± 5.5        | 3512 ± 34      | 40.6 ± 0.7         | -3.7 ± 0.2                  | 3.1 ± 0.1             | -7.2 ± 0.4               |
| Dif.    | 0.9 ± 1.2         | 89 ± 27        | 1.6 ± 1.2          | 0.1 ± 0.1                   | 0.1 ± 0.1             | 0                        |

ONL, outer nuclear layer; ROS + IS, rod outer + inner segment; V max, b-wave maximum amplitude; STR, scotopic threshold response; R, luzindole + DMSO; L, DMSO; Dif., difference between right and left eye; cd, candela. Average and control values are ± SEM.

that retinal melatonin receptors play a role in modulating light-damage susceptibility.

MATERIALS AND METHODS

Animals

These studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male Sprague-Dawley albino rats were obtained from Charles River Laboratories (Wilmington, MA) at 7 weeks of age. Before experiments began, the rats were housed in our colony room for at least 7 days in 50 lux illumination (VitaLite) on a 12-hour light/dark cycle with lights on at 7:00 AM. They were fed a high-fat breeding chow (Formulab; PMI Nutrition International, Inc., St. Louis, MO).

Drug and Injection

Luzindole (N-acetyl-2-benzyltryptamine) was obtained from Tocris Cookson, Inc. (model N-0774; Ballwin, MO). In initial experiments with four animals we used phosphate-buffered saline (PBS) as the injection vehicle, but then we switched to 50% dimethyl sulfoxide (DMSO; Sigma Chemical Co., St. Louis, MO) for the remainder of the animals. Because luzindole does not dissolve well in PBS, this mixture remained cloudy. One microliter of a 1 mM luzindole solution was injected into the vitreous of the right eye using a Hamilton syringe and 30-gauge needle, and 1 µl vehicle (either PBS or DMSO) was injected into the left eye of each animal as a control. Injections were made with the unaided eye through the sclera behind the limbus under dim red light from a small flashlight. A small air bubble was injected along with the solution that could be observed in the vitreous and used to confirm that the solution had been injected properly. The procedure took less than 1 minute for each eye, with the total exposure to dim red light (<5 lux) not exceeding 5 minutes. Shortly after the injection, we observed the eye for signs of hemorrhage or cloudiness; if either occurred, the animal was not used. Of 11 rats injected, only 2 were rejected. Injections were performed between 3 PM and 5 PM, and the rats then were kept in darkness until the onset of bright light exposure at 10:00 AM the following day.

Light Exposure

Rats were between 8 and 9 weeks of age at light exposure. At this age, rats are sexually mature and begin to show greater susceptibility to light damage, which continues to increase until at least 16 to 24 weeks of age. Our earlier experiments had indicated that rats of this age and origin suffered substantial light damage after 24 to 48 hours' exposure to moderate intensities of light (1000–3000 lux). Although animals from the
After this preparation, they were dark adapted for an additional 12 hours before recording ERGs. The animals were prepared under dim red light, and both pupils were dilated with 0.1% atropine and 0.1% phenylephrine HC1. Animals were adapted for 12 hours before recording ERGs. The animals were adapted for a nominal light intensity, but interocular differences between the electroretinograms (ERGs) of animals exposed to the same nominal light intensity, but interocular differences within the same animal were not significant.

Food and water were provided ad libitum during exposure, and the temperature was kept at 22.5 ± 1°C. After light exposure, rats were returned to the colony room under normal cyclic lighting for 7 days. Two age-matched un.injected control rats were maintained in the colony room under standard cyclic light of 50 lux.

**ERG Recordings**

Seven days after bright light exposure, animals were dark adapted for 12 hours before recording ERGs. The animals were prepared under dim red light, and both pupils were dilated with 0.1% atropine and 0.1% phenylephrine HCl. Animals were anesthetized intramuscularly (IM) with a loading dose of xylazine (10 mg/kg) and ketamine (14 mg/kg) and then maintained by IM infusion via pump (Razel Instruments, Stamford, CT). After this preparation, they were dark adapted for an additional 30 minutes. Animals were held still by a bite bar and nose clamp during recordings, and a heating pad maintained body temperature at 36.8°C.

ERGs were recorded bilaterally using silver wire loops on the cornes of each eye, with 1% tetracaine topical anesthesia and a drop of methylcellulose to maintain corneal hydration. Silver wire reference electrodes were placed on the nasal sclera 1 mm from the limbus of each eye. A subcutaneous ground wire was inserted on the scalp. Responses were amplified at 10,000 gain from 0.1 Hz to 1000 Hz, filtered to remove 60-Hz noise, and digitized at a rate of 50 kHz on two channels. ERGs were processed with Adobe Photoshop software, version 4.0 (Adobe Systems, Inc., Mountainview, CA).

**RESULTS**

One day after ERG recordings, the rats were killed with an overdose of sodium pentobarbital, and the eyes were removed for retinal morphology, using overnight fixation with 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4°C. Eyes were trimmed and postfixed in 1% osmium buffer at 4°C. Epon-embedded tissue was cut into 1-μm sections and stained with toluidine blue for light microscopy. All sections for light microscopy were cut along the vertical meridian of the eye passing through the optic nerve.

Histology

One day after ERG recordings, the rats were killed with an overdose of sodium pentobarbital, and the eyes were removed for retinal morphology, using overnight fixation with 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4°C. Eyes were trimmed and postfixed in 1% osmium for 1 hour. Epon-embedded tissue was cut into 1-μm sections and stained with toluidine blue for light microscopy. All sections for light microscopy were cut along the vertical meridian of the eye passing through the optic nerve.

Data Analysis

An initial study on four rats (luzindole in PBS) focused primarily on ERG results, and preliminary histologic assessment of two of these rats measured outer nuclear layer (ONL) thickness by counting rows of cells at a single location in the inferior retina. In the second study, we treated five rats with luzindole in DMSO and more extensive histologic assessment was used, including ONL thickness and cell count and the combined width of the rod outer-segment and inner-segment layers (ROS + IS) at multiple points along the inferior and superior halves of retinal sections. Ten measurements spaced 400 μm apart were made in each retinal half beginning 400 μm from the optic nerve. The total number of ONL cells was counted in 100-μm lengths of retina in these 20 positions. Michon et al. showed that ONL thickness measurements correlated well with counts of single photoreceptor nuclei. We included both methods because of a concern that ONL thickness might not be as reliable in severely damaged regions of the retina. Photomicrographs were taken with an image capture system consisting of a video camera (Optronics Engineering, Goleta, CA) on an Olympus Vanox microscope (Tokyo, Japan), and images were processed with Adobe Photoshop software, version 4.0 (Adobe Systems, Inc., Mountainview, CA).

ERG b-wave amplitude was plotted against light intensity on log-log plots to determine b-wave threshold and b-wave maximum amplitude. STR threshold was readily determined by inspection of the raw waveforms. ONL cell counts are reported as totals, and thicknesses of the ONL and ROS + IS layers as the average of the 20 locations along each section.

The statistical analysis was designed to overcome possible differences in the extent of light damage among animals by testing the null hypothesis that the treated right eye was not different from the control left eye for each animal. The right eye–left eye difference was computed for each animal for the ERG and histology measures, and the average was determined across the treated animals. The difference from zero (i.e., the null hypothesis) was determined by Student’s two-tailed t-test.

**RESULTS**

As described in the Materials and Methods section, animals 1 to 4 were involved in initial studies using PBS as the vehicle, and 5 to 9 were studied more extensively with DMSO vehicle. The outcomes from both sets of animals were not different, but the results that we present here are primarily from animals 5 through 9 (Table 1).

Severe photoreceptor damage occurred in the light-exposed eyes and resulted in the loss of most of the ONL photoreceptor nuclei. Despite the severe damage, a substantial amount of protection was seen in eyes injected with luzindole. The photomicrograph in Figure 1 illustrates this protective effect in one animal. As expected from other studies, damage was most severe in the sensitive region, which lies in the superior central retina approximately 1.5 mm from the optic nerve and is known to be the most susceptible to light-induced photoreceptor damage. In the vehicle-injected control eye, very few photoreceptor nuclei remained in this sensitive region, and no outer segments were apparent (Fig. 1A). In contrast, the ONL in the sensitive region of the luzindole-treated eye is 3 to 4 nuclei thick, and short but intact ROS are seen. This suggests that luzindole enhances some rod maintenance.
A Luzindole Treated

DMSO Control

B Luzindole Treated

DMSO Control

FIGURE 1. Photomicrographs of the treated and control retinas from animal 5 at two different locations. (A) The “sensitive region” in the superior retina where light damage is most severe. Only an occasional photoreceptor nucleus is seen in the dimethyl sulfoxide (DMSO) injected control eye, and the outer plexiform layer and inner nuclear layer (inl) are directly opposed to the retinal pigment epithelium (pe). The outer nuclear layer (onl) of the luzindole-treated eye is 3 to 4 nuclei thick, and rod outer-segment layers (ros) are still present but shortened. (B) The corresponding region in the inferior retina where the damage is less severe; the outer nuclear layer thickness differs by 1 to 2 nuclei, and the rod outer-segment layer shows greater density and better structure in the luzindole-treated eye.

By contrast, animal 6 to 9, which are in Table 1, were much more severely damaged. Figure 2 is a plot of the distribution of cell loss for animal 6 and shows that substantial protection occurred throughout the inferior retina. In the superior retina, however, which had the most severe damage, although protection is seen at most locations except immediately adjacent to the optic nerve, the difference between the two eyes was much smaller than in the inferior retina. This suggests that very severe damage overcame some of the protection in the luzindole-injected eye.

ERG measurements were performed on these animals to evaluate how well function was preserved in luzindole-treated eyes. The ERG intensity series in Figure 3A is from the first experiment (animals 1-4) in which PBS was used as the vehicle. In the luzindole-treated eye, a small b-wave is seen rising out of the STR trough at -2.8 log cd/m² (Fig. 3A), but in the vehicle-treated eye, the b-wave is detectable at approximately 0.5 log cd/m², giving a 3.3-log unit difference. The b-wave intensity-response curves for animal 6 (Fig. 2 and Table 1) and an animal not exposed to damaging light are shown in Figure 3B. The log-log curve for the luzindole-treated eye is similar to that of the normal animal but is shifted down.
approximately 1.5 log units higher than that of the control rat and photoreceptor cells, the luzindole-treated eye reaches the damage is more severe than in the retina shown in Figure 1.

The width of the ROS + IS layer was about three times greater damage both in terms of histologic and ERG changes. ONL and to the right, indicating a threshold sensitivity reduction and reduced b-wave maximum ($V_{\text{max}}$). Despite an 80% loss of photoreceptor cells, the luzindole-treated eye reaches the 10-$\mu$V criterion threshold amplitude at an intensity only approximately 1.5 log units higher than that of the control rat and has a b-wave $V_{\text{max}}$ approximately 1 log unit lower. However, in the vehicle-injected eye, the increase in threshold was $>4$ log units greater than in the luzindole-treated eye, and the b-wave $V_{\text{max}}$ was reduced nearly 2 log units further. This large functional difference between the eyes is consistent with the histologic findings, of a much better preserved ROS structure and a greater number of photoreceptor cells, in the luzindole-injected eyes.

Table 1 summarizes the results with luzindole/DMSO-treated eyes in five rats, 5 to 9, most with relatively severe damage both in terms of histologic and ERG changes. ONL thickness and ONL cell count both showed significant preservation in the luzindole-injected eyes compared with the vehicle-injected eyes ($P < 0.001$). ONL cell counts and ONL average thicknesses in the light-damaged retinas were tightly correlated with each other in luzindole-injected ($r = 0.98$) and vehicle-injected ($r = 0.91$) eyes. The extent of protection by luzindole, expressed as the ratio of the values of the treated right eye to those of the control left eye, was similar for both.

The width of the ROS + IS layer was about three times greater in the luzindole-treated eyes. We also noted that the extent of protection, expressed by this ratio and when compared across animals, increased as the overall damage in the control eye increased. A similar correlation of protection to degree of injury has been shown before in unilateral light-damage protection by optic nerve section. In the present study this effect was highly linear for animals with the most severe damage (rats 6 to 9) for ONL cells ($r = 0.994$) and ERG b-wave threshold ($r = 0.953$). This indicates that much of the variability in the extent of rescue across animals is due to the variability in damage severity.

Light-damage elevated the b-wave threshold in the vehicle-injected eyes by an average of approximately 5.6 log units above undamaged control eyes (Table 1). However, in luzindole-treated eyes the b-wave threshold was altered by only approximately 2.5 log units, illustrating substantial preservation of function compared with vehicle-injected eyes. A similar result was seen for maximum b-wave amplitude. There was a small but significant difference in STR threshold. As in the Royal College of Surgeons (RCS) rat, light damage the STR threshold was affected much less than the b-wave by photoreceptor cell degeneration. Overall, in animals 1 to 9 from both experiments (see the Methods section), the average b-wave threshold was $2.7 \pm 0.5$ log cd/m$^2$ lower ($P < 0.001$), the maximum b-wave amplitude was $1.0 \pm 0.2$ log $\mu$V higher ($P < 0.001$), and the STR threshold was $0.5 \pm 0.1$ log cd/m$^2$ lower ($P < 0.005$), in the luzindole-treated eyes.

**DISCUSSION**

These results show that intravitreal injection of the competitive melatonin receptor antagonist luzindole conveys protection from light-induced photoreceptor damage in the rat. Substantial preservation of physiological function (shown by ERG measurements) and a moderate degree of photoreceptor cell rescue (by cell counts and ONL thickness) were observed after a single intravitreal injection of the antagonist before light exposure. These results suggest that endogenous melatonin can modulate light-damage susceptibility in the rat by binding to retinal melatonin receptors. The highest density of retinal melatonin receptors in the mammal is in the inner plexiform layer. Thus, it seems possible that modulation of light-damage susceptibility by luzindole and melatonin receptors may be mediated by mechanisms that involve neurons postsynaptic to photoreceptors.

It is known that endogenous melatonin suppresses dopamine release from amacrine cells at night. Suppression of dopamine release by melatonin occurs via GABAergic ($\gamma$-aminobutyric acid) neurotransmission, and this suppression can be blocked by luzindole, which enhances the release of dopamine in the dark. Bromocriptine, a dopamine D2 agonist, conveyed protection against photoreceptor light damage in rats when administered systemically, whereas melatonin enhanced the damage. There is considerable evidence of dopamine receptors on photoreceptor cells and for an influence of dopamine on many aspects of photoreceptor metabolism (see Refs. 21 and 22 for review). The dopamine receptors on the photoreceptor cells are of the D2/D4 subtype, which are negatively coupled to cAMP formation. These observations have led to speculation that the effect of melatonin on light-damage susceptibility is mediated by decreased dopamine release and by decreased occupation of dopamine receptors on photoreceptor cells. Together with the evidence that luzindole increases the release of retinal dopamine in the dark, our study lends additional support to this hypothesis.

A possible link between light-damage protection by optic nerve section and retinal dopamine was postulated by Bush and Williams. They suggested that a loss of centrifugal regulation of retinal dopamine release could have been responsible for the reduced light-damage susceptibility they observed after...
A Right eye (luzindole in PBS) Left eye (PBS)

![Graph A](image)

**B**

![Graph B](image)

**FIGURE 3.** (A) Dark-adapted electroretinogram (ERG) waveform series for the luzindole-treated right eye, and phosphate-buffered saline (PBS), vehicle-treated left eye. Both eyes were recorded simultaneously from one animal in our initial experiment. Flash intensity is given on the left side. STR, scotopic threshold response. (B) ERG b-wave response versus intensity data for animal 6 and for an undamaged control animal maintained in dim cyclic light plotted on a log-log scale. DMSO, dimethyl sulfoxide.

optic nerve section. More recent evidence indicates that centrifugal fibers terminating in the outer retina of the rat are indoleamine-accumulating and that they may serve as a source of serotonin for melatonin synthesis by photoreceptors. 

Along with the present study linking melatonin receptors in the retina to light-damage susceptibility, this evidence supports a role for the melatonin-dopamine system in the protection afforded by optic nerve section.

Another possible cellular target for luzindole that could influence photoreceptor survival is the retinal pigment epithelium (RPE). Melatonin inhibits cAMP formation in cultured rat and human RPE cells. This effect apparently is mediated by pertussis toxin-sensitive melatonin receptors. If melatonin has a similar action in vivo, it could influence ROS disc shedding and other aspects of the interaction between the RPE and photoreceptor cells. One of these is the regeneration of the visual pigment, which requires isomerization of the chromophore in the RPE. Because light damage is thought to be mediated via light absorption by rhodopsin, it is possible that luzindole affected rhodopsin content or regeneration kinetics during light damage through an effect on the RPE. This possibility could be investigated in a future study by determining the level of steady state rhodopsin bleach and regeneration during light-damage exposure. Bush and Williams used this technique to show that an alteration in rhodopsin content or kinetics was not a factor in light-damage protection by unilateral optic nerve section.

Compared with the histologic differences between the luzindole- and vehicle-treated eyes, the difference in ERG parameters was much larger and more variable (Table 1). The ONL thickness and ROS + IS length, for example, were about two and three times larger, respectively, in luzindole-treated eyes compared with control eyes. On the other hand, the ERG b-wave threshold and amplitude were different in the two eyes by 1 log cd/m² and 2.5 log cd/m², respectively. Part of this is undoubtedly due to normal relationships between photoreceptor cell number and rhodopsin content and the ERG parameters. Rapp and Williams reported that a linear change in whole-retina rhodopsin content by light damage caused a logarithmic change of b-wave threshold, and we have noted similar results for ERG parameters versus ONL cell count. This suggests that small histologic effects in light damage can have large functional ERG consequences. Additionally, however, there may have been some specific rescue of function in the remaining cells beyond what was apparent by either cell number or ROS length. Measuring retinal rhodopsin content may allow a better correlation between the degree of cell rescue and ERG function, because both are whole-retina parameters.

In this study, we have seen that light-damage effects, particularly as measured by the ERG, are quite variable between animals (but not between eyes of the same animal) despite fairly uniform lighting in our light-damage box. We observed this variability in another study in which untreated animals were exposed to a range of light intensities to compare the correlation between morphometric and ERG parameters. This is a common finding in light-damage experiments, both in terms of retinal sensitivity and morphometric measurements. One possible reason for a variability in light-damage susceptibility is because there is a variability in light history during animal rearing. This could certainly be a factor in our experiments because levels of in-cage illumination at the supplier are unknown and are likely to be quite variable. Because we compared one eye to the other in the same animal, interanimal variability should not have had a significant impact on our results. It could, however, contribute to the variations in rescue effect because the degree of rescue increased with increasing overall damage. It has been noted by others and others that if both eyes in the same animal are untreated or if both receive control injections, they show a very similar degree of light damage. In addition, we carefully checked
animals before and after light exposure to make sure that the ocular media were clear in both eyes. Another reason for variability in light damage is animal behavior during exposure, such as closing or covering the eyes. Williams et al. recently showed that lid closure in albino rats could reduce effective bleaching of rhodopsin by 50%. We monitored the animals frequently during exposure and saw no behaviors that might result in more light exposure to one eye than the other.

In summary, these results indicate that luzindole can ameliorate light damage. One of the prime objectives of future studies will be to determine whether dopamine receptors are involved in this protection. Recently, A₂A-adrenergic agonists and pre-exposure to bright light before light damage were shown to decrease susceptibility to retinal light damage. Both of these treatments caused the expression of bFGF to increase in the retina. bFGF itself decreases light-damage susceptibility when administered intravitreally. Thus, we plan to determine whether the administration of luzindole is accompanied by increased bFGF expression. We have recently observed, however, that exposure even to very dim red light (10 lux) shortly before light damage can convey protection (authors' unpublished observations) even though one would not expect this to raise bFGF levels. Rescue by very dim light is more consistent with previous studies that have suggested that light can act through retinal neurohormones or neuromodulators to enhance photoreceptor survival.

References


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