Functional Rescue of Photoreceptors From the Damaging Effects of Constant Light by Survival-Promoting Factors in the Rat

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Purpose. To investigate whether and how survival-promoting agents rescue photoreceptor cell function and morphology from constant light damage, the authors recorded electroretinographic (ERG) responses and examined light micrographs of retinas in those rats given intravitreal injection of midkine (MK) and basic fibroblast growth factor (bFGF) before constant exposure.

Methods. Albino Sprague-Dawley rats were injected with MK, bFGF, or phosphate-buffered saline (PBS) 2 days before the onset of 1 week of constant light. ERG responses were recorded using white flash stimuli with the intensity range of 4 log units, followed by histologic examinations of retinas, including quantitative assessment of the outer nuclear layer thickness as an index of photoreceptor cell loss.

Results. ERG responses were barely detectable in un.injected eyes after 1 week of constant light. On the other hand, distinct responses were recordable in eyes injected intravitreally with MK and bFGF, and the degree of ERG rescue in terms of the amplitude of b-wave was approximately 40% to 60% compared with normal eyes. Intravitreally injected PBS showed slight, but noticeable, preservation of ERG responses as well. Histologic examination revealed that MK and bFGF protected photoreceptors from light damage. A good correlation was found between anatomic rescue of photoreceptors as assessed by outer nuclear layer thickness and the functional rescue as defined by the magnitude of ERG responses.

Conclusions. Functional and anatomic rescue of photoreceptors in albino rats from constant light damage is achieved by prior intravitreal injection of MK and bFGF.


Growth factors and neurotrophic agents have survival-promoting activity in the retina. LaVail and others1-4 demonstrated that basic fibroblast growth factor (bFGF) injected into vitreous cavity or subretinal space delays the inherited photoreceptor degeneration in Royal College of Surgeons rats and that intravitreal administration of multiple growth factors, including bFGF and neurotrophic agents, rescues photoreceptors from constant light-induced or ischemia-induced retinal degeneration in albino rats. Midkine, a heparin-binding growth factor, also was found by Unoki et al5 to have the rescue effects of photoreceptors from the damages of constant light. The photoreceptor rescue effects by survival-promoting agents were shown by light microscopic examinations of the retina to reveal distinct preservation of the photoreceptor outer and inner segments and maintenance of the thickness of the outer nuclear layer,1-4 but these studies do not provide information on the functional survival of photoreceptor cells. Does the anatomic rescue of photoreceptor cells also correspond to preservation of retinal function? Does any functional preservation occur in correlation with the degree of morphologic rescue of photoreceptor cells? To answer these questions, we studied whether and how electroretinographic (ERG) responses are protected from constant light damage by intravitreally injected (midkine) MK and bFGF in albino rats, together with subsequent histologic examination of tested retinas. Midkine is a recently defined heparin-binding growth factor produced by a retinoic acid-responsive gene and expressed in the retina as well as in neural tissues,6 and its intravitreal injection has been shown to protect photoreceptors significantly from constant light-induced cell death in the albino rat.7 We report herein evidence that MK and bFGF have functional and anatomic survival-promoting activity in the retina.

MATERIALS AND METHODS. Animals. Sprague-Dawley albino rats 2 to 3 months of age (Kyudo, Kuma-moto, Japan) were reared in cyclic light (12 hours on/12 hours off, with an in-cage illuminance of approximately 15 foot-candles) for more than 7 days before use. All experimental procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Kagoshima University Faculty of Medicine Guidelines for Animals in Research.

Factors Injected. Midkine was purified from the culture medium of L cells transfected with an MK expression vector by the methods described else-

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where, and it was concentrated to 1 μg/μl. Human recombinant bFGF (1 μg/μl) was commercially available (R & D Systems, Minneapolis, MN). The control vehicle was phosphate-buffered saline (PBS).

Injection and Constant Light Exposure. Two days before exposure to constant light, animals were anesthetized with an intramuscular injection of a mixture of ketamine (84 mg/kg)-xylazine (6 mg/kg), and they were administered a single 1-μl solution of MK or bFGF in the vitreous cavity of one eye and a single 1-μl solution of PBS in the contralateral eye. The injections were performed with a 32-gauge beveled needle through the sclera, choroid, and retina at a point midway between the ora serrata and the equator of eyeball. Two days after intravitreal injection, the animals were exposed for 1 week to constant light at an illuminance of 130 to 150 foot-candles.

Electroretinographic Procedure. After 1 week of constant light, uninjected rats or those injected with survival-promoting factors or PBS, as well as those reared in cyclic light, were examined for ERG response using methods elsewhere. The animals were anesthetized with an intraperitoneal administration of 1500 mg/kg of urethane, and they were kept dark adapted for more than 15 minutes in an electrically shielded, darkened room at a temperature of 30°C. Before ERG recordings, pupils were dilated with drops of 1% tropicamide, and eyes were kept open by lid traction threads. ERG response was recorded with a cotton wick differential electrode placed on the conjunctiva and an indifferent electrode positioned over the skin between the eyes, elicited by white flashes of 5-msec duration produced with a 500-W xenon arc, and varied in intensity by the insertion of neutral-density filters. Stimuli were produced at increasing intensity in steps of 1 log unit over a range of 4 log units. Ten responses for each stimulus intensity were recorded, averaged by a computer (ATAG-150, Nihon Kohden; Tokyo, Japan), and plotted on an X-Y plotter, as described elsewhere. An adequate interval between stimuli was allowed to maintain a constant level of dark adaptation—a 5-second interval for the three dim flashes and a 50-second interval for the two bright flashes.

Measurement of ERG responses was performed under masking for treatment regimens: a-wave amplitude from the baseline to the bottom of the negative wave, b-wave amplitude from the bottom of a-wave to the peak of b-wave, and b-wave peak time from the stimulus onset to the peak of b-wave.

Histologic Procedure. After ERG recordings, eyes were enucleated and retinas were examined by light microscopy according to methods described elsewhere, which included measurements of the thickness of outer nuclear layer.

RESULTS. ERG and Photoreceptor Damage After 1 Week of Constant Light in Uninjected Rats. Cyclic light-reared, normal eyes showed distinct ERG responses consisting of relatively small a-waves and prominent b-waves with oscillatory potentials, the magnitudes of which varied systematically, with flash stimuli covering an intensity range of 4 log units (Fig. 1A). In contrast, ERG responses were highly depressed in eyes after 1 week of constant light without prior injection (Fig. 1B). These uninjected eyes showed nonrecordable-type responses even to the maximum stimuli available—barely detectable b-wave responses with prolonged peak time.

After ERG responses were recorded, the retinas of uninjected eyes were examined by light microscopy. Degeneration of photoreceptor cells was as expected and showed marked loss of photoreceptors with thin outer nuclear layers (Fig. 1B).

ERG Rescue After Intravitreal PBS Injection. PBS-injected eyes showed a marked reduction in ERG responses, but they behaved differently from uninjected eyes. These eyes showed measurable responses that amounted to as large as 100 μV of b-wave with the maximum stimulus, indicating occurrence of a certain degree of functional preservation of photoreceptors by PBS injection. The ERG preservation by PBS (P-MK; = P-bFGF) injection was evaluated by the ratio of b-wave amplitudes to those in normal eyes, and it was approximately 20% when determined for a collection of responses elicited by various stimulus intensities (Fig. 2).

ERG and Photoreceptor Rescue After Intravitreal MK and bFGF Injection. Intravitreally injected MK and bFGF showed protective effects of photoreceptors from light damage electroretinographically as well as morphologically. Figure 2 illustrates overall ERG results describing the amplitude of b-wave as a function of log relative stimulus intensity. Intravitreal injection of MK before constant light was significantly more effective than PBS injection in the preservation of ERG responses (Fig. 1C). MK-injected eyes demonstrated invariably larger b-wave responses than PBS-injected contralateral eyes for the whole stimulus intensities. The degree of ERG rescue was defined by the ratio of b-wave amplitudes elicited by various stimulus intensities to those in normal eyes, and it was more than 40% (Fig. 2). Although quantitative assessment of the a-wave of ERG responses was not attainable because of the much smaller magnitude of responses, it also appeared to be rescued by intravitreal MK (Fig. 1C).

Prior injection with bFGF protected ERG from constant light damage, demonstrating a marked preservation of the b-wave in the whole range of stimulus intensities. The ratio of the mean amplitude of b-wave
FIGURE 1. Electroretinographic (ERG) records and light micrographs in Sprague-Dawley rats with various treatments. (A) Normal eye reared in cyclic light. (B) Eye after 1 week of constant light without prior injection. (C) Eye with an intravitreal injection of midkine (MK) 2 days before the onset of 1 week of constant light. (D) Eye with an intravitreal injection of basic fibroblast growth factor (bFGF) 2 days before the onset of 1 week of constant light. Left panel illustrates ERG responses; each trace represents an average of 10 responses elicited by various flash stimuli. These records demonstrate a marked depression of ERG in uninjectected eyes for all intensities of stimuli and a significant rescue of ERG from light damage by intravitreal MK and bFGF. Right panel illustrates light micrographs, each of which was obtained from the eye corresponding to the ERG panel. (A) Normal retina shows numerous outer and inner segments with 9 to 10 rows of photoreceptor nuclei. (B) The constant light-exposed retina shows loss of outer and inner segments of photoreceptors with a marked decrease in thickness of the outer nuclear layer composed of only 1 to 3 rows of nuclei. (C) The MK-injected retina shows rescue of photoreceptors with preservation of outer nuclear layer consisting of 5 to 6 rows of nuclei. (D) The retina rescued by bFGF, with features similar to that injected with MK. Hematoxylin-eosin. Bar = 20 μm. RPE = retinal pigment epithelium; OS = outer segment of photoreceptors; IS = inner segment of photoreceptors; ONL = outer nuclear layer.

FIGURE 2. Electroretinographic (ERG) response in Sprague-Dawley rats with various treatments. The amplitudes of b-wave are plotted as a function of log relative flash intensity. □ = Cyc L, cyclic light-reared, normal eye; ■ = CL, eye receiving constant light without injection; △ = MK, eye injected with MK; ▲ = P-MK, eye injected with phosphate-buffered saline (PBS) whose fellow eye was injected with MK; ○ = bFGF, eye injected with PBS; ● = P-bFGF, eye injected with PBS whose fellow eye was injected with bFGF. Each data point was obtained from a single eye. The degree of ERG rescue was defined by the ratio of b-wave amplitude in MK and bFGF for collected data by high intensities, and it was calculated to be: MK = 0.42 ± 0.25 (mean ± SD, n = 9); P-MK = 0.19 ± 0.09 (n = 9); bFGF = 0.61 ± 0.31 (n = 9); P-bFGF = 0.15 ± 0.04 (n = 9). The degree of ERG rescue by MK and bFGF is significantly larger than that by PBS (paired t-test, P < 0.005). MK = midkine; bFGF = basic fibroblast growth factor.

was as large as 60% of normal eyes (Fig. 2). The a-wave also was preserved significantly, but its quantitative assessment was not feasible because of its smaller amplitude.

Histologic examination showed virtually the same features as those in previous similar experiments, revealing significant protection of visual cells from constant light damage. The degree of light-induced retinal degeneration was quantified by measuring outer nuclear thickness and comparing with the magnitude of ERG b-wave responses in the corresponding eye (Fig. 3). There was a significant relationship between the degree of photoreceptor cell damage or rescue between outer nuclear layer thickness and magnitude of ERG response: The more the outer nuclear layer was preserved, the larger was the response the ERG elicited.
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FIGURE 3. Anatomic and functional rescue of photoreceptor cells by intravitreal growth factors, midkine (MK) and basic fibroblast growth factor (bFGF), from constant light damage in Sprague-Dawley albino rats. The abscissa represents thickness of the outer nuclear layer, as measured by the methods described elsewhere, and the ordinate indicates the amplitude of ERG b-wave elicited by maximum and 1.0 log unit less intense stimuli. Each data point was obtained from a single eye. □ = cyc L, cyclic-reared, normal eyes; ■ = CL, eyes exposed to constant light without injection; Δ = MK, eyes injected with MK; ▲ = P-MK, eyes injected with PBS whose fellow eye was injected with MK; ○ = bFGF, eyes injected with bFGF; ● = P-bFGF, eyes injected with PBS whose fellow eye was injected with bFGF. There is a significant relationship between the thickness of the outer nuclear layer and the amplitude of the b-wave, so that when more photoreceptor nuclei are preserved, larger ERG responses are produced. The sigmoid curve is empirically drawn to be best fitted to the data point and describes the relation between outer nuclear layer thickness b-wave amplitude (correlation coefficient = 0.925, n = 32).

DISCUSSION. These results indicate that constant light produces a marked reduction in ERG activity and that the degree of its depression corresponds to that of photoreceptor degeneration in albino rats. Constant light has been shown, and confirmed in the current study, to affect predominantly the outer retina.2,3 In the ERG a-wave is thought primarily to reflect photoreceptor activity, and the b-wave is thought to be generated by Müller cells to interact with synaptic transmission. Thus, the marked damage of ERG a- and b-wave by constant light is as expected from the anatomic changes in the outer retina.

New information available from the current experiments is that intravitreally injected MK and bFGF protect ERG activity from constant light damage. There was a significant correlation between the magnitude of ERG response as evaluated by b-wave amplitude and the preservation of photoreceptor cells as assessed by outer nuclear layer thickness. Hence, the survival-promoting agents tested herein provide protective or rescue effects of photoreceptors from light damage not only anatomically but also functionally. Although no such studies were performed, in view of the association between retinal electrical activity and visual sensation, it is likely that animals pretreated with MK and bFGF will have discernible preservation of behavioral visual function after constant light exposure. The functional rescue was, however, incomplete, and it was estimated to be approximately 40% to 60% of the normal function. Its degree would be enhanced with modification of the treatment regimen, including modification of dosage and time of administration of survival-promoting factors. The bFGF-treated eyes demonstrated significant regeneration of photoreceptors in the 10-day cyclic light recovery after light damage and almost normal structure in the regions of best regeneration.2

An additional finding is that intravitreal injection of PBS also preserved ERG activity as well as photoreceptor morphology, although the degree of rescue was significantly less than that of survival-promoting agents. Similar observations have been reported that the intravitreal or subretinal injection of PBS, or the insertion of a dry needle, results in a marked protective effect of photoreceptors in the RCS rat1 and in the albino rat.2 The current experiments emphasize that subtle injury may provide rescue of photoreceptor cells functionally and anatomically.

The mechanism by which MK and bFGF protect photoreceptors from constant light damage remains to be elucidated. Midkine is a recently defined heparin-binding growth factor that promotes neurite outgrowth and survival of embryonic neurons in culture.1,8,10 This growth factor is a product of retinoic acid-responsive gene, and it has been shown to be expressed intensely in the retina as well.11 The intraretinal synthesis of retinoic acid by amacrinc and Müller cells, and the presence in retinal neurons and Müller cells,12,13 suggest an important role in the maintenance and organization of the retina. In any event, MK and bFGF may have roles in the maintenance of neural tissues, including the retina.

Many growth factors, cytokines, and neurotrophic agents other than bFGF and MK have been shown to protect or rescue photoreceptor cells from inherited retinal degeneration1 and constant light- or ischemic injury-induced retinal degeneration.2,5 Although
those agents are suggested to have therapeutic potential, the degree of anatomic rescue of photoreceptors is variable among agents. Further studies are warranted to assess survival-promoting agents for the preservation of retinal function, whereby recording of ERG responses provides a relatively simple, fast, reliable test.

Key Words
basic fibroblast growth factor (bFGF), electroretinographic response, light damage, midkine, rat, retinal degeneration

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Clinical Evaluation of Multifocal Electroretinogram
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Purpose. To compare the multifocal electroretinogram (ERG) system to conventional gan/feld and focal ERGs obtained from patients with known retinal diseases to assess its clinical applicability.

Methods. A multi-input system analysis was used to explore the field topography of ERG responses to local luminance modulation in patients with retinitis pigmentosa, pericentral pigmentary retinal dystrophy, branch retinal arterial occlusion, or idiopathic macular hole.

Results. The dysfunctional areas measured by multifocal ERG were compatible with those assumed by combined findings of gan/feld and focal ERGs. However, the wave shapes of multifocal ERG in the retina with arterial occlusion differed from those of conventional focal ERG, suggesting that the negative and positive deflections shown in the first-order kernel of multifocal ERG may not correspond to conventional a- and b-waves of ERG.


Although many authors have applied focal electroretinogram (ERG) to detect focal retinal abnormalities, the averaging technique used to improve the signal-to-noise ratio permits testing of a single local...