Time Course of Neurotrophic Factor Upregulation and Retinal Protection against Light-Induced Damage after Optic Nerve Section

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PURPOSE. To assess neurotrophic factor upregulation in the retina after damage to the optic nerve and relate that regulation to changes in photoreceptor stability and function.

METHODS. Retinas of adult pigmented (Long-Evans) rats were examined at successive times (1–60 days) after unilateral optic nerve section. The distribution and expression of ciliary neurotrophic factor (CNTF) and basic fibroblast growth factor (FGF-2) and their receptor elements FGFR1 and CNTFRα were studied with immunohistochemistry and Western blot analysis. FGF-2 and CNTF mRNA levels were also assessed, with semiquantitative reverse transcription-PCR. Levels and localization of the intracellular signaling molecule ERK and its activated, phosphorylated form pERK, were examined by immunohistochemistry. To assess the correlation between neurotrophic factor levels and their protective effect against light damage, albino (Sprague-Dawley) rats were exposed to bright continuous light (1000 lux) for 24 or 48 hours at successive times after nerve section. The TUNEL technique was used to visualize neuronal cell death in the retina.

RESULTS. CNTF upregulation was detected 1 week after optic nerve section, peaked at 2 weeks, and fell to control levels at 4 weeks. CNTF appeared first in the inner retina in the ganglion cells, then in the Müller cells in which it became prominent at the outer limiting membrane (OLM) and in the outer segment (OS) region of photoreceptors. FGF-2 upregulation became prominent, particularly in photoreceptors, 21 to 28 days after surgery, continued to 2 months, and slowly declined thereafter. Double labeling with antibodies to ligand and the receptor showed colocalization of CNTF to its receptor at the OS region, whereas FGF-2-to-FGFR1 binding was found in the outer nuclear (ONL) and outer plexiform (OPL) layers. Optic nerve section provided a significant protective effect against light-induced damage in the first 2 weeks. There was no protection when animals were exposed to damaging light 1 month after nerve section.

CONCLUSIONS. The upregulation of CNTF 7 to 14 days after nerve section correlates with a reduction in the a-wave described previously. Colocalization of CNTF and CNTFRα on the outer segments suggests that CNTF acts at the photoreceptor membrane. The slower upregulation of FGF-2 correlates with a reduction of the b-wave. FGF-2/FGFR1 colocalization in the OPL suggests that this factor acts at the synaptic terminals of photoreceptors, modulating the release of neurotransmitters. The time course of pERK upregulation suggests that the successive upregulation of CNTF and FGF-2 activates the ERK pathway. Based on the time course of protection against bright continuous light, it seems that CNTF plays a major role in this effect, and FGF-2 has a less important role in the protection against light-induced damage. (Invest Ophthalmol Vis Sci. 2005;46:1748–1754) DOI:10.1167/iovs.04-0657

Damage to the optic nerve causes retrograde degeneration of retinal ganglion cells, but also induces changes in the cell biology of the retina, including its outer layers. In particular, nerve section increases the resistance of photoreceptors to damaging light, the expression of the trophic factor FGF-2, in photoreceptor somas and the expression of the cytokine CNTF in Müller cells and is followed by a decrease in the amplitude of the a-wave (photoreceptor) and the b-wave (postsynaptic) component of the ERG (Cervetto et al. IOVS 2001;42:Abstract 3396). Previously, we3 presented evidence that several of these effects can be accounted for by an upregulation of FGF-2 levels in the retina. The effect of nerve section of the b-wave, for example, is mimicked by intraocular injection of FGF-2, and FGF-2 and its receptor FGFR1 are found in the synaptic terminals of photoreceptors, specifically on synaptic vesicles within those terminals.7 It has been clear for some time, however, that stress regulates the expression of several trophic factors and cytokines in the retina7 and evidence has emerged recently8 that different components of the ERG are affected at different times after the section of the optic nerve. To test whether different factors have differential effects on retinal function, we tested the working hypothesis that the regulation of CNTF and FGF-2 after nerve damage may differ in time course and that the difference may relate to differences in regulation of the a- and b-wave of the ERG (see Ref. 5). Herein, we present evidence that the time course of upregulation induced by nerve section differs between CNTF and FGF-2 and that the differences correlate well with ERG changes reported previously5,9 in, respectively, the a- and b-waves of the ERG.

METHODS

All experiments were performed in compliance with the Animal Experimentation Ethics Committee guidelines and in accordance with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. Experimental animals were adult Long-Evans pigmented rats, born and raised in 12-hour dark-light cyclic light, with the light cycle...
set at 200 lux. In experiments involving light-induced damage, animals were adult Sprague–Dawley albino rats, born and raised in 12-hour dark-light cyclic light set at 5 to 10 lux.

**Unilateral Optic Nerve Section**

Nerve section was performed in rats under surgical anesthesia by intraperitoneal injection of either tribromoethanol (270 mg/100 g, Avertin; Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) or a mixture of ketamine (100 mg/kg) and xylazine (12 mg/kg). The surgical approach to the optic nerve has been described. In brief, the skin was incised at the superior rim of the orbit, and the orbital tissue behind the eye was blunt dissected to visualize the optic nerve. The nerve was cut several millimeters behind the eyeball, to avoid damaging vessels entering or leaving the eye. The skin was closed with sutures, and an antibiotic/suppressant powder applied to the wound. At various times after surgery, animals were euthanatized and eyes were collected for processing.

**Light-Induced Damage**

Animals were placed in individual transparent Plexiglas cages, with food available on the floor and water provided from clear plastic bottles, both ad libitum. A light source placed above the cages generated an intensity of 1000 lux at eye level. Animals were dark adapted overnight and then exposed to the bright light continuously for 48 hours. Exposure started at the same time of day for all animals (9 AM). The rats were euthanatized at the end of the exposure, and eyes were enucleated and immersion fixed in 4% paraformaldehyde for further processing.

**Immunohistochemistry**

Immediately after euthanasia, eyes were enucleated and immersion fixed in 4% paraformaldehyde for 1 hour, washed in 0.1 M phosphate-buffered saline (PBS, pH 7.4), and cryoprotected by immersion in 15% sucrose overnight. Eyes were embedded in optimal cutting temperature (OCT) compound (Tissue Tek; Sakura Fintek, Torrance, CA), snap frozen in liquid nitrogen–isopentane and cryosectioned at 20 μm. Each eye was oriented so that sections ran from the superior to the inferior edge. Sections were collected on gelatin- and poly-l-lysine-coated slides. They were then immunolabeled for the following proteins: CNTF (Chemicon, Temecula, CA 1:200, FGF-2 (Upstate Biotechnology, Lake Placid, NY) 1:200, CNTFRα (RDI, Flanders, NJ) 1:50, extracellular signal-regulated kinase (ERK) 1:50, phosphorylated ERK (pERK) 1:100 (Cell Signaling Technology, Beverly, MA), and FGF1 1:100 (Santa Cruz Biotechnology Inc., Santa Cruz, CA). For all labeling, washes were performed three times for 5 minutes each in 0.1 M PBS at room temperature. To block nonspecific binding, 10% normal horse serum was used. Sections were incubated with antibodies at 37°C for 70 minutes. Antibodies against CNTF, FGF1, ERK, and pERK were rabbit polyclonals, the antibody against CNTFRα was a goat anti-rat polyclonal, and the antibody against FGF-2 was mouse monoclonal. Secondary antibodies were goat anti-mouse, goat anti-rabbit, or donkey anti-goat IgG conjugated to Alexa Fluor 488 (green) or 594 (red) diluted 1:200 in PBS (Molecular Probes, Eugene, OR) and incubated at 37°C for 1 to 2 hours, followed by three 5-minute washes in PBS, and counterstaining with DNA-specific label, bisbenzamide (Calbiochem, La Jolla, CA) 1:10,000 for 1 minute at room temperature (RT). Images were taken by laser scanning microscope (Leica, Deerfield, IL) and analyzed with NIH image (ImageJ 1.14c for Linux platform; available by ftp at zippy.nimh.nih.gov/ or at http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). Measurements of FGF-2 labeling were made across the full thickness of the ONL in the midperipheral retina (approximately half-way between the disc and the superior edge). Measurements of CNTF-labeling were made over an area covering the thickness of the INL, to include the most strongly CNTF-labeled elements in the retina, the somas of Müller cells.

**TUNEL Labeling**

To label apoptotic cells, we used the TUNEL technique, according to earlier published methods.

**Western Blot Analysis**

Isolated rat retinas were washed in Western blot buffer (50 mM Tris-HCl [pH 7.4], 50 mM NaCl, and 5 mM EDTA) and homogenized in a lysis buffer (0.2% Triton X-100, protease inhibitor [Sigma-Aldrich Chemie GmbH], and 5% DNase diluted 1:100 in wash buffer). Two retinas were pooled per sample, and homogenates were incubated in the lysis buffer for 30 minutes at RT. After incubation, samples were centrifuged at 15,000g for 20 minutes at 4°C. The resultant supernatant was collected, and protein content was measured (Protein Assay; Bio-Rad, Munich, Germany). Supernatant was diluted in an equal volume of 2× Laemmli buffer (Sigma-Aldrich Chemie GmbH), boiled for 5 minutes and stored at −20°C until all samples were collected. Protein of each sample (100 μg) was electrophoresed on a 15% SDS-polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane using transfer buffer (25 mM Tris-HCl [pH 8.3], 192 mM glycine, and 20% methanol). The protein blot was then blocked with 3% nonfat dried milk and 0.05% Tween 20 in blot buffer (20 mM Tris HCl [pH 8.0], 500 mM NaCl) at RT for 45 minutes. The membrane then was incubated overnight at 4°C with the rabbit anti-rat CNTF antibody (Chemicon), or the anti-FGF-2 clone bFM-2 mouse monoclonal IgG (Upstate Biotechnology) diluted 1:1000, or the anti-pERK mouse monoclonal (Santa Cruz Biotechnology Inc.) diluted 1:200 in the blocking buffer. The reactions were visualized with horse-radish peroxidase-conjugated secondary antibodies (anti-rabbit or anti-mouse IgGs) for 2 hours at RT. Bands were visualized and quantified using a chemiluminescence method (ECL Western blot system; Amer sham Pharmacia Biotech, Freiburg, Germany).

**Reverse Transcription–Polymerase Chain Reaction**

Ten samples for each series were collected and total RNA was extracted (TRizol reagent; Invitrogen-Life Technologies, Scotland, UK) according to the manufacturer’s directions. Total RNA (1 μg) was reverse transcribed (RETROscript; Ambion, Cambridge, UK) with random decamers used as first-strand primers. The cDNA (2 μL) was used as the template for amplification in a 20-μL PCR reaction. PCR was performed in duplicate and in multiplex reactions, using 18S as the internal standard (QuantumRNA Classic 18S; Ambion), producing a 489-bp PCR product. The 18S rRNA internal control is used in combination with 18S competitors and a gene-specific PCR primer pair. Competimer technology was used to modulate the amplification efficiency of a PCR template, and the correct ratio between the 18S RNA primers and 18S competitors was empirically determined. The sequences of the FGF-2-specific primer pair were: forward, GGA GTG TGT GCT AAC CGT TAC CTG GC AT ATG; reverse, TCA GCT CTT AGC ACC AGA CAT TGG AAG AAA AAG—and produced an amplification product of 242 bp. The product of the CNTF primers was 1050 bp and the sequences of the primers were: forward, CAC ATT TCT TAT TGG GAC; reverse, ACA ACA ATA CTC TTT TTA TTC. The number of cycles was determined empirically by sampling FGF-2 and CNTF amplitcons between 22 and 40 cycles and selecting the approximate midpoint of the linear amplifications (35 cycles). PCR thermal cycling for FGF-2 mRNA amplification and classic 18S was performed under the following conditions: initial denaturation for 10 minutes at 94°C; followed by 35 cycles of 40 seconds at 94°C, 40 seconds at 65°C, and 1 minute at 72°C; and a final extension of 5 minutes at 72°C. The multiplex amplification parameters for CNTF and classic II 18S were: 2 minutes and 30 seconds at 94°C (initial denaturation), 1 minute at 94°C, 1 minute at 50°C, 1 minute at 72°C for 35 cycles and a final extension of 5 minutes at 72°C. PCR products were separated on a 2% agarose gel stained with ethidium bromide, and the relative densities of the PCR fragments were determined and normalized using software for semiquantitative densitometric analysis (Total Laboratory, Phoretix, Newcastle-upon-Tyne, UK). Results are given as relative units (RU).
RESULTS

Time Course of CNTF Upregulation after Nerve Section

CNTF Protein Levels and Cellular Localization. Immunolabeling for CNTF was relatively nonspecific in control retinas (Fig. 1A, control), with the label appearing to surround somas in the INL and, more weakly, the ONL. After section (Figs. 1A, 1–28 days), the specificity of labeling increased, showing the somas of Müller cells in the INL from 1 day and the radial processes of Müller cells crossing the ONL and forming the OLM, from 7 days. This localization of CNTF predominantly in Müller cells confirms previous descriptions\textsuperscript{4,11,12} and persisted to the longest survival examined. By inspection, labeling appeared brightest and most extensive at 14 days. Intensity analysis of images of immune-labeled tissue showed maximum labeling at 14 days (Fig. 1C). Western blot analysis did not detect significant difference in protein levels from the control at any of the time points studied after the nerve was sectioned (data not shown).

CNTF mRNA Changes. RT-PCR analysis detected two increases in mRNA levels after nerve section (Fig. 2A, 2C, 2D), one at 2 to 6 hours after surgery, and the second at 15 days.

FGF-2 Protein Levels and Cellular Localization. In control retinas, as described previously,\textsuperscript{7,11,13,14} FGF-2 was apparent in ganglion cell somas, and in Müller cell bodies in the INL (Fig. 1B, control). After nerve section, no significant change in labeling intensity or localization was apparent until day 21, when labeling appeared in photoreceptors in the ONL (Fig. 1B, 21 day PL [post lesioning], arrow). This labeling of ONL somas was initially prominent at the inner margin of the layer. By 28 days, FGF-2 labeling of ONL somas was prominent and extended through most of the layer. Quantitatively, the brightness of immunolabeling for FGF-2, measured across the full thickness of the ONL, show a limited increase at 7 days, and a progressive increase from 14 to 21 to 28 days (Fig. 1C). Similar trends were noted in three experimental series, performed in different laboratories. The trend shown in Figure 2C is representative of all series.

Western blot analysis was also performed, and results are shown in Figure 1D. The antibody used labeled two distinct fragments (22–24 kDa and 18 kDa). The intensity of labeling showed a time course similar to that obtained with immunolabeling, with the major increase in FGF-2 protein levels delayed to the 28-day time point.

FIGURE 1. Expression of FGF-2 and CNTF protein in rat retina at successive times (1–30 days) after optic nerve section. (A) CNTF immunolabeling of the ONL (o) and INL (i). Arrows: level of the OLM. (B) FGF-2 immunolabeling of the ONL and INL. Arrow: regions where FGF-2 was upregulated in photoreceptor somas in the ONL. (C) Measurements of CNTF: (red) and FGF-2: specific immunofluorescence (green), as a function of time after optic nerve section. CNTF-fluorescence, as measured over the thickness of the INL, and FGF-2-fluorescence over the full thickness of the ONL. (D) Top: Western blot analysis for FGF-2 protein expression in retinas before (C), and 7 to 30 days after optic nerve section. The labeling distinguished two fragments (18 and 22–24 kDa) of the FGF-2 protein. Bottom: the density of labeling for the two fragments.
CNTF mRNA and 242-bp sequence of FGF-2 mRNA. (A, B) Subscripts indicate negative control (c−) and positive control (c+) lanes, and mRNA from control rats (C) and from rats examined at 1 to 48 hours, and 4 to 30 days after lesioning. (C, D) Density of labeling in the gels in (A) and (B) on two different time scales (hours, days).

FGF-2 mRNA Changes. RT-PCR analysis detected an early increase in FGF-2 mRNA levels 2 to 6 hours after optic nerve section. After this time, mRNA levels returned to control levels, increased to 4 days, and then returned to control levels (Figs. 2B, 2C, 2D). The persistence of high FGF-2 protein levels after the transient upregulation of FGF-2 mRNA suggests that the protein is stabilized, by a still unknown mechanism.

Site of Action of Neurotrophic Factors: Receptor Localization

CNTFRα. In other tissues, CNTF has been shown to act through a tripartite receptor complex. CNTF binds to the α component of the complex, but that component does not have a cytoplasmic domain and plays no role in the signaling process.15-16 The binding of the CNTF to its specific α subunit initiates the formation of a complex, by the recruitment of the β components LIFβ and gp130 and the formation of a heterodimer of these two components, which then initiates the signaling process by activating either cytoplasmic tyrosine kinases,15 or ERK members of the MAP-kinase cascade.17

As previously described,12 we found punctate labeling of CNTFRα along Müller cell processes across the retina, but the most prominent labeling was on the photoreceptor outer segments (Figs. 3A-C, green). This localization of CNTFRα on photoreceptors18 or more specifically on its outer segments19 has been confirmed. At 7 days and most clearly at 14 days, CNTF labeling (Figs. 3A-C, red) was upregulated, as evidenced by the prominent yellow signal of the outer segment labeling. The yellow signal suggests colocalization of the ligand and receptor, as demonstrated previously.12

FGFR1. In control retinas, FGFR1-labeling (Figs. 3D-F, red) was prominent in somas in the ONL, where it was more intense near the OPL.7 By 21 days after nerve section, the expression of FGF-2 in the ONL increased, particularly in the cytoplasm of ONL somas. Yellow staining of these somas was prominent at 1 and 2 months after nerve section, specifically in the ONL (Figs. 3E, 3F). Again, the yellow staining suggests colocalization of the ligand and receptor, as demonstrated previously.7 Figures 3G and 3H show ONL somas labeled for both FGF-2 and FGFR1, in control material (Fig. 3G) and 1 month after nerve section (Fig. 3H). Again, yellow staining, indicative of ligand/receptor colocalization, is more prominent after nerve section (bottom panels Figs. 3G, 3H).

Mode of Action of Neurotrophic Factors in the Retina: Intracellular Signaling Using the ERK Pathway after Optic Nerve Section

Cytokines act through the Jak-STAT pathway, but an increasing body of evidence is showing that the MAPK pathway is also likely to be involved in mediating their effects.20 Growth factors act by phosphorylation and activation of the ERK family, also known as MAP kinases (MAPKs).20-25 Activation of these kinases involves their translocation to the nucleus and phosphorylation; the phosphorylated form then activates transcription factors. Since the MAPK pathway may be involved in both cytokine and growth factor signaling, we investigated changes in levels of the phosphorylated (active) form of ERK (pERK) induced by optic nerve section, at key times (7, 14, 21, and 28 days) after lesioning; in six experimental series, three examined by immunohistochemistry (Figs. 4A-E), and three by Western blot analysis (Figs. 4F, 4G).

All series showed a bimodal upregulation of pERK over the period examined (0–30-day after lesioning). In control retinas, immunolabeling for pERK was not prominent in any retinal layer (Fig. 4A). One week after nerve section, pERK labeling was strongly present in Müller cell bodies in the INL, and along the full length of their processes from the OLM to the ILM, where astrocytes were also labeled. Horizontally oriented processes were also labeled in the OPL and IPL. In the IPL they appeared to mark the sublamine of the layer (Fig. 4B). Labeling was reduced 14 days after nerve section (Fig. 4C) and was upregulated again at 21 days (Fig. 4D). One month after optic nerve section the intensity of labeling was reduced again, being prominent in only some Müller cell bodies (Fig. 4E).

Western blot analysis showed increases in pERK protein levels at 7 and 30 days after nerve section (Figs. 4F, 4G). The two forms of analysis are thus consistent in showing a bimodal upregulation of pERK, with an early peak at 7 days after lesioning and a sharp reduction at 14 days. The time of the second peak was at 21 day in the immunohistochemical analysis (Fig. 4E) but at 30 days or later in the Western blot analysis.

Correlation with Light Damage

Exploration of the effects of nerve section on trophic factor expression in the retina began with the observation7 that nerve section is followed by a period of photoreceptor resistance to light-induced damage. To confirm this observation and define its time course we exposed nerve-sectioned rats to bright continuous light (BCL: 1000 lux for 48 hours) at successive intervals after nerve section and assessed the photoreceptor death induced, using the TUNEL technique. Four animals were examined at each survival time (0, 7, 14, and 30 days). As previously,14 BCL induced the DNA fragmentation characteristic of dying cells almost exclusively in the photoreceptor layer (Fig. 5A). The rate of photoreceptor death induced by BCL, expressed as the frequency of TUNEL+ profiles/mm of retina, decreased with time to a minimum (~50% of control) at 7 days after lesioning (Figs. 5A, 5B) and recovered monotonically thereafter (Fig. 5B). The minimum at 7 days after lesioning corresponds to a rise in the expression of CNTF. We observed no minimum in BCL-induced death that corresponded with the peak upregulation of FGF-2, at ~30 days after lesioning.
FIGURE 3. Effect of optic nerve section on ligand and receptor binding of CNTF and FGF-2. (A–C) CNTF and CNTFRα on the outer segments (OS). In control retinas, CNTFRα labeling (green) was dominant. At 7 days, and more prominently at 14 days, CNTF-labeling (red) appeared and colocalized with CNTFRα labeling (yellow). o, nuclei of photoreceptors (blue) in the ONL. (D–F) FGF-2 and FGFR1 labeling of the retina. o, ONL; i, INL; g, ganglion cell layer. In control retina (D), FGF-2 was prominent in Müller cell somas in the INL and in ganglion cell somas. FGFR1 labeling (red) was prominent in the ONL and in ganglion cell somas whose cytoplasm was labeled for both FGF-2 and FGFR1 (yellow). At 30 days (1 month) and at 60 days (2 months) FGF-2 (green) was upregulated in the ONL. The FGF-2 labeling colocalized with FGFR1 labeling, in the ONL (yellow). In each of (D–F), the red signal has been removed over a central portion of the image, to show the labeling for FGF-2. The scale in (F) refers to (D, E, G, H). Top in (G). FGF-2 (green) and FGFR1 labeling (red) of the OPL (opl) and surrounding layers. Bottom: only the yellow pixels in the top panel, where red and green pixels colocalized. Colocalization was evident in somas in both the ONL and INL, but not in the OPL. At 30 days (H, 1 mo) after nerve section, colocalization was more prominent in somas of both layers, and was also detected in the OPL (arrows). The scale in (H) refers to (A, B, C, G).

DISCUSSION
The present study documents the impact of damage to the optic nerve on the cell biology of the retina, in particular on the expression of neuroprotective proteins, specifically the growth factor FGF-2 and the cytokine CNTF. The retina reacts to nerve damage by upregulating these proteins, whose binding with their receptors increases, activating intracellular ERK pathways and protecting photoreceptors against light stress.

Trophic Factors and Retinal Function
We have recently described a strong correlation between ERG response amplitude and trophic factor expression in the rat retina. A detailed analysis of the a-wave after optic nerve section suggested a reduced dark current at 1–2 weeks after nerve section, temporally related to an upregulation of CNTF. Although the molecular events leading to a reduced ion current have to be clarified, they may be triggered by the binding of CNTF ligand to its receptor CNTFRα on the photoreceptor outer segment. The reduced amplitude of the b-wave is related to the a-wave reduction for up to 21 days after section, and subsequently seems to be due to postsynaptic events triggered by FGF-2 upregulation.

Value of the Nerve Section Model of Retinal Stress
Continued attention to this model seems warranted for two reasons. First, the retina’s reaction to damage to the optic nerve resembles in many ways its reaction to light-induced damage, mechanical injury, or hyperoxic injury. In the nerve section model, however, the photoreceptors are free of damage, restricted to the retrograde degeneration of ganglion cells, located in the inner retina. In other stress models (light-induced damage, hypoxic or hyperoxic damage, mechanical damage), by contrast, the photoreceptors are directly affected. In the nerve section model uniquely, the photoreceptors are protected but undamaged. First, the present results show that the upregulation of CNTF and FGF-2 induced by nerve section follow distinct time courses. The nerve section model thus presents an opportunity to test photoreceptor stability when CNTF is upregulated and FGF-2 is not (e.g., at day 7 after lesioning) and vice versa (day 28).

Protection against Light Damage: Is FGF-2 Important?
The present results confirm the original report of Bush and Williams that nerve section induces an increase in photoreceptor resistance to damaging light, and the report of Kostyk et al. that nerve section induces an upregulation of FGF-2 in photoreceptors. When we traced the time course of the increase in photoreceptor resistance, however, the result was unexpected: Resistance correlated with the upregulation of CNTF and not of FGF-2. Many studies have reported a correlation between upregulation of FGF-2 in the retina/photoreceptors and photoreceptor resistance to stress. In most, however, FGF-2 upregulation was induced by injury to the retina (needlestick, laser injury, pre-conditioning light challenge), which upregulated a range of potentially protective factors (reviewed in Ref. 32). Consider...
ing the detail of those studies, those that give evidence of an FGF-2-specific protection of photoreceptors against damaging light are LaVail et al. and Faktorovich et al. They used subretinal injection of FGF-2, and control experiments (subretinal needle insertions) showed that much of the effect resulted from the impact of the injection. LaVail et al. used intravitreal injection of FGF-2, which arguably involves less retinal damage and still showed significant FGF-2-related photoreceptor rescue from light damage. One other FGF-2-specific study deserves note: Campochiaro et al. showed that dominant-negative competition for FGF-2 in transgenic mice caused photoreceptor degeneration, indicating an important role for FGF-2 in photoreceptor stability. They did not, however, test whether dominant negative competition for FGF-2 affects vulnerability to light-induced damage.

The present evidence seems to be the first to question the relationship between FGF-2 and the resistance of photoreceptors to damaging light. The present data do not however question the role of FGF-2 in photoreceptor stability in normal lighting conditions, or in the face of other forms of stress, such as hyperoxia, hypoxia, or genetically induced. We are currently using the nerve section model to assess the roles of CNTF and FGF-2 in these forms of stress.

**Correlation with Light-Induced Damage: A Particular Role for CNTF?**

Conversely, the present results suggest a particular role for CNTF in the protection of photoreceptors against damage by light. Many previous studies have shown a general correlation between CNTF upregulation and photoreceptor resistance to damage, and one study has shown a CNTF-specific effect. Recent studies of the retinal distribution of CNTF (the element common to the family of CNTF receptors; reviewed in Refs. 16,35) have shown that CNTF is prominent in the outer segments of photoreceptors, and that CNTF/CNTFRα colocalization occurs on outer segments and is upregulated in light-stressed retina. The available evidence seems to suggest, therefore, that CNTF plays a major role in increasing photoreceptor resistance to light-induced damage, perhaps by acting at the outer segment level. The present study did not monitor factors such as BDNF

**Figure 4.** Expression of pERK in the retina after optic nerve section. (A–E) Immunolabeling for pERK across the rat retina. Labeling was low in a control retina (A), and became prominent in the IPL and OPL and in the somas and radial processes of Müller cells at 7 days and again at 21 day after lesioning. o, ONL; i, INL; ipl, IPL. (F) Western blot for pERK protein. Bands for 42- and 44-kDa fragments of pERK were prominent at 7 days and again at 30 days after nerve section. (G) Density of 42- and 44-kDa bands in Western blot analysis (F) for up to 30 days after lesioning.

**Figure 5.** Changes in photoreceptor vulnerability to damaging light after optic nerve section. (A) Sections of retina from rats exposed to bright light for an extended period (BCL; 1000 lux for 48 hours). Blue: nuclear DNA of cells in the INL (i) and ONL (o), labeled with bisbenzamide. Red: fragmentation of DNA induced by BCL, labeled with the TUNEL technique. Labeling was intense in control animals (0 days' exposure) and again at 30 days. TUNEL labeling was consistently reduced (i.e., photoreceptor vulnerability was reduced) at 7 days. DNA fragmentation was specific to the ONL and therefore to photoreceptors. (B) The data in black show BCL-induced TUNEL labeling of the ONL, expressed as TUNEL profiles/mm and averaged over the full length of retinal sections, for successive times after nerve section. Exposure to BCL was for 48 hours. Vulnerability was minimal at 7 days and significantly (P < 0.0001 on a 2-tailed t-test) lower than in control subjects. By 30 days, vulnerability had returned toward control levels (P = 0.2, by two-tailed t-test). The red and green curves show the time course of expression of FGF-2 and CNTF over the same period.
and IL-1β, which have also been shown to protect photoreceptors from light-induced damage when injected intravitreally. The reduction of retinal responsiveness associated previously with CNTF upregulation suggests, however, that the protection does not extend to photoreceptor performance, and our own observations in the nerve-sectioned rat confirm this suggestion. In two strains of mouse in which the retina degenerates, the rd and rds strains, CNTF upregulation has reported to be associated with an improvement in the ERG. A subsequent study of one of these strains reported, however, that upregulation of CNTF expression using an AAV-mediated gene delivery system negated the effects of gene therapy on retinal function, as assessed by the ERG. Further, the same upregulation of CNTF expression in wild-type mice resulted in a marked reduction of the ERG.

In summary, the present results suggest that CNTF upregulation plays a major role in the protection of photoreceptors and reduction of the ERG which follow damage to the optic nerve. The linkage between increased resistance of photoreceptors to damage and the loss of their responsiveness to light, first reported by Gargi et al., deserves further investigation.

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