Encapsulated Cell-Based Intraocular Delivery of Ciliary Neurotrophic Factor in Normal Rabbit: Dose-Dependent Effects on ERG and Retinal Histology

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PURPOSE. ERG and histologic changes were investigated in normal rabbits after intravitreal implantation of encapsulated cell technology (ECT) devices releasing ciliary neurotrophic factor (CNTF).

METHODS. Fifteen adult New Zealand White albino rabbits had ECT devices secreting CNTF at 22, 5, or 0 ng/d implanted in the superior temporal quadrant of the left eye. The low dose has been shown to produce substantial rescue of photoreceptors in the rd11 mouse model of retinal degeneration. Both eyes were untreated. Ganzfeld dark- and light-adapted ERGs and clinical observations were performed at 5, 15, and 25 days after implantation. Rod a-waves and rod and cone b-waves and outer nuclear layer (ONL) morphology were evaluated at 25 days.

RESULTS. Clinical examination showed minimal changes in a few CNTF-treated eyes, including vitreous membranes and engorgement of iris vessels at day 25. Retinas appeared normal. CNTF did not significantly affect the rod a- or b-waves, although the b-wave amplitude tended to be larger in CNTF-treated retinas at low flash intensities. The cone b-wave amplitude was significantly reduced in high-dose eyes at some flash intensities. The ONL area in high-dose eyes was significantly greater because of increased thickness than in fellow retinas. ONL cell size was significantly increased, and staining density decreased in CNTF-treated retinas.

CONCLUSIONS. CNTF, given by intravitreal ECT device at doses that protect photoreceptors in a canine model of retinal degeneration (5 ng/d), did not adversely affect either rod or cone ERG function of normal rabbit retina. The cone ERG was more sensitive to suppression being reduced, at low flash intensities, by 22 ng/d. Dose-related changes in the ONL and photoreceptor cell nuclei did not represent a toxic effect, because they were not associated with deficits in the rod ERG over a broad range of intensities. (Invest Ophthalmol Vis Sci. 2004;45: 2420–2430) DOI:10.1167/iovs.03-1342

CNTF (ciliary neurotrophic factor) rescues neurons in degenerative diseases in animal models1,2 and may be a promising nonspecific therapeutic in inherited retinal degenerative conditions. Recent experiments demonstrated that CNTF protected against photoreceptor cell loss in retinal degeneration models in mouse and rat.11–12 However, an important goal is development of a safe and effective means of chronic delivery of CNTF. Several previous studies used long-term, viral-vectored delivery of CNTF to the retina in mouse11,12 or rat13. Unfortunately, it is difficult to regulate the level and the site of CNTF expression with viral gene transfer techniques. The vector delivers the gene to a number of cell types, and the gene product is expressed intracellularly rather than interacting with receptors on the external cell membrane. In addition, several of the previous studies were suspected of delivering toxic dose levels of CNTF.

Encapsulated cell technology (ECT) provides extracellular delivery of CNTF through continual and stable intraocular release at known doses through a device implanted in the vitreous chamber. CNTF delivered by ECT produced dose-dependent photoreceptor rescue in the rd11 mouse model of retinal degeneration, but retinal function was not evaluated in that study. We implanted ECT devices releasing CNTF in the eyes of normal rabbits to study the effects of exogenous CNTF, at appropriate therapeutic dose levels, on normal retinal function. Because previous studies have shown morphologic changes in the outer nuclear layer (ONL) related to CNTF gene transfer,11 we also evaluated retinal histology. CNTF released at 5 ng/d in the vitreous did not diminish the rabbit rod or cone ERG, but, in fact, tended to increase the rod b-wave amplitude near threshold. A higher dose of CNTF (nominally 22 ng/d) did not diminish the rod ERG, but it produced a small reduction of the cone ERG. Both doses resulted in measurable changes in ONL morphology, indicating that the threshold for these effects is lower than that for the effects of CNTF on the ERG.

METHODS

Animals

The study was conducted in accordance with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. Fifteen New
Zealand White rabbits, weighing 2.0 to 2.2 kg (4–5 weeks of age), were housed in separate cages under a 12-hour light–dark cycle with food and water available ad libitum. Albino rabbits were chosen, because ocular pigmentation may protect against drug toxicity. 14

**ECT Intraocular Implantation**

ECT devices were provided by Neurotech USA (Lincoln, RI) and were kept in a carbon dioxide cell culture incubator (37°C) until intravitreal implantation. The 10-mm long, 1-mm diameter device is a sterile, nonpyrogenic, retrievable implant specially developed to study treatment of retinal degenerative diseases. 9 It contains human RPE cells genetically modified to secrete recombinant human CNTF. The device consists of a sealed semipermeable membrane capsule surrounding a scaffold of six strands of polyethylene recombinant CNTF. The device allows the outward diffusion of growth factors and other cellular metabolites and the inward diffusion of nutrients necessary to support the cell survival in the vitreous cavity while protecting the contents from host cellular immunologic attack. The CNTF delivery rate of the device was determined before implantation and after retrieval from the rabbit eye by ELISA (R&D Systems, Minneapolis, MN). Rabbits were randomly divided into three groups, with five animals in each group, for implantation of ECT devices with different CNTF delivery rates (as determined post explant): the empty devices (containing no cells) and the low-dose (nominally 5 ng/d) and high-dose (nominally 22 ng/d) CNTF implants. The low dose device had already shown efficacy in rescuing cell numbers in a canine model of retinal degeneration. 9 The high-dose devices were included to evaluate possible toxicity of higher levels of CNTF in the vitreous. The experiment was masked, and the researchers were not informed about the groups until the end of the experiment.

Rabbits were anesthetized intramuscularly with ketamine (25 mg/kg) and medetomidine (0.5 mg/kg), positioned on their sides on the operating table, and given supplemental oxygen through a nose cone during the entire procedure. The ECT devices were implanted into left eyes under an operating microscope through a 2-mm scleral incision parallel to and approximately 4 mm posterior to the limbus. The device was positioned in the superior temporal quadrant with the tip toward the center of the vitreous cavity and fixed to the sclera with 6-0 nylon suture. The scleral and conjunctival incisions were sutured closed. No damage to the lens or retina was observed after the surgery. The right eyes were not operated on.

The surgical wounds were checked daily for the first week and every other day afterward until the end of the experiment. Rabbits were given topical ocular gentamicin and prednisolone ointment twice daily for 10 days. One rabbit in the high-dose group died under anesthesia during ERG recording at day 15 after implantation. The data from this rabbit were excluded.

The devices were explanted from the implantation site, without complication, using the same surgical procedure. At the end of the explantation procedure, animals were euthanatized with an overdose of pentobarbital sodium (Beuthanasia-D solution; Schering Plough Animal Health, Omaha, NE). The eyes were enucleated and processed for histologic examination. Devices were assayed within 24 hours for CNTF output.

**Clinical Ophthalmic Observation**

Ophthalmic examinations were conducted before ECT implantation and every 5 days after surgery. The anterior segment examinations included the cornea, anterior chamber, iris, and lens. The vitreous, retina, and the ECT implant were observed by indirect ophthalmoscopy. Fundus pictures were taken from two representative rabbits from each of the three groups at 24 days after implantation.

**ERG Recording**

Standard Ganzfeld dark- and light-adapted ERG signals were recorded at 5, 15, and 25 days after ECT implantation. The rabbits were dark-adapted for at least 1 hour before recording and were anesthetized 20 minutes before ERG recording with a mixture of ketamine (25 mg/kg) and xylazine (2 mg/kg). The cornea was anesthetized with topical 0.5% proparacaine HCl, and pupils were fully dilated with 1% tropicamide. Burian-Allen corneal bipolar electrodes (Hansen Laboratory, Iowa City, IA), containing both reference and active electrodes integrated into a contact lens-type electrode, were used, and the ground was placed subcutaneously on the back. The rabbit was placed on its stomach on a board, with its head resting in the natural upright position. The whole front part of the animal could then be slid inside a large Ganzfeld bowl. Both eyes were recorded simultaneously.

Signals were amplified at 10,000 gain and band-pass filtered between 0.1 and 1000 Hz. Stimulus intensity was set by neutral density (ND) filters at 0.5-log-unit steps covering a 6.0-log-unit range up to 2.0 log cd/m². The photopic ERG was recorded on a continuous light-adapting white background of 34 cd/m² with stimulus intensity beginning near the photopic b-wave threshold at −1.0 log cd/s/m².

**ERG Analysis**

Scotopic (dark-adapted) and photopic (light-adapted) a- and b-wave peak amplitudes were measured from the preresponse baseline or, in the case of the b-wave when an a-wave was present, from the a-wave maximum. Because the cone a-wave was relatively small at these flash
intensities, it was not used in this study. ERG data were analyzed on computer (nlme library, ver. 3.1-38; in R, ver. 1.6.2, http://www.r-project.org/; developed by Robert Gentleman and Ross Ihaka, University of Auckland, New Zealand and provided in the public domain by the R Project for Statistical Computing; and PROC MIXED in SAS for Windows; ver. 8.0.2, SAS Institute, Inc., Cary, NC). The primary outcome variables were log of the ratios between treated and untreated eyes for a- and b-wave amplitudes at 25 days after implantation. Predictor variables were ERG stimulus intensity and treatment group. Random effects for rabbit and slope of regression line were included in the model. These random effects implicitly adjust for correlation between measurements obtained from individual rabbits.

Retinal Histology

Enucleated eyes were fixed with Davidson’s fixative for 24 hours and then rinsed with tap water and transferred to 10% neutral buffered formalin for shipment to a commercial histology laboratory (Research Pathology Services, New Britain, PA) where the eyes were processed for paraffin embedding, sectioned vertically through the pupillary optic nerve (superior-inferior), and stained with hematoxylin and cosin. The average ONL width in every 500-μm length of retina (from one to three measurements made within a 500-μm length on each section) was determined for each experimental group of rabbits. Measurements were made on digital images taken with a microscope (model E800; Nikon, Tokyo, Japan) and digital camera (DXM 1200). The total ONL area in each retinal section was calculated from the length and width measurements that were made by an observer masked to the experimental groups. Measurements on randomly selected images were verified by additional observers.

ONL cell counts were performed on digital images taken at high power (130-μm fields) and processed in image analysis software (Photoshop ver. 6.0; Adobe Systems, Mountain View, CA). A grid with 12 × 1-μm squares was overlaid on each image. The number of photoreceptor nuclei in six alternate squares was counted. The average was multiplied by the number of squares occupied by the ONL to obtain total cells in that field. Counting was done at two locations in the superior peripheral retina: 100 and 1500 μm from the ora serrata.

**Figure 2.** ERG amplitude versus flash intensity for the dark-adapted a- and b-waves in the empty-device (A), low-dose (B), and high-dose (C) eyes compared with the control eyes in each group. For comparison of experimental groups all eyes implanted with devices are plotted on the same graph in (D). Error bars, SD.
Photoreceptor cell size (area) and staining density were measured at the same locations on images collected and processed in the same way and then transferred to image-management software (Scion Image for Windows; Scion Corp., Frederick, MD). The images were converted to grayscale (255 steps), and the photoreceptor nuclei in alternate grid squares (10–25 nuclei per eye) were outlined using the drawing tool, which, once calibrated, allows the program to measure area and average pixel density. To be selected for measurement, the margin of the cells, either membrane or chromatin, had to be easily distinguished and not touching the grid lines or another cell. Overlapping cells were not measured. These procedures were performed by a single observer who was masked to the experimental group of each retina. No attempt was made to distinguish rod and cone nuclei.

RESULTS

Clinical Observations

After surgery all rabbits had normal corneas, anterior chambers, and clear lenses. Six rabbits (three each from low- and high-dose groups) had a minimal vitreous membrane at day 25, and two rabbits (one each in the low- and high-dose groups) showed iris vascular engorgement at day 25. No vitreous hemorrhage or retinal lesion was observed clinically.

Dark-Adapted ERG

Dark-adapted ERG waveforms and amplitudes of ECT device-implanted eyes were comparable to those of control eyes in all groups, over a wide range of intensities. However, there were small differences in amplitude between implanted and control eyes in some rabbits at high flash intensities (Fig. 1). Plots of ERG amplitude versus flash intensity (V-log I) at 25 days (Fig. 2) confirm that the average responses were comparable across all groups and between control and experimental eyes, but indicated possible effects of ECT devices at some stimulus intensities. In both empty-device and high-dose groups, a small reduction in amplitude of the b-wave in implanted eyes, relative to their respective control eyes, was observed from moderate intensities, near the b-wave plateau, to high intensities.
sections, from the superior to the inferior ora serrata (Fig. 8C).

By 25 days, both high-dose and empty-device animals showed b-wave amplitude ratios below 1 at high stimulus intensities, consistent with the V-log I data and suggesting the reduction was not due to CNTF. Applying the statistical model to the overall difference in dark-adapted b-wave ratios at 25 days indicated no significant effect of treatment \((P = 0.07)\). However, there was a significant interaction of intensity and treatment \((P < 0.0001)\), indicating the treatments had different effects at different intensities and justified a point-wise comparison. The ratio in animals with CNTF secreting devices was above 1 at the lowest three intensities but lower than the empty-device animals from day 15 onward. The difference was statistically significant at day 25 \((P < 0.05\) and \(P < 0.0005)\). This suggests there may have been enhanced sensitivity of the b-wave in CNTF-treated eyes at these intensities. By contrast, the a-wave ratio in the high-dose and low-dose groups was consistently below 1 and was below the empty-device group at 5 days. However, this effect was transient, and by 25 days, there was no statistical effect of treatment on the a-wave \((P = 0.61)\).

Light-Adapted ERG

The ERG in the presence of a rod-suppressing background light reflects activity of the cone pathway, primarily cones and cone bipolar cells. The “cone” ERG in all implanted eyes had timing and waveforms comparable to control eyes. However, the b-wave amplitudes in high-dose ECT eyes were smaller than in the control, as seen in the waveforms at 25 days in Figure 4. On average, high-dose eyes tended to have reduced b-wave amplitudes relative to control eyes, much of the lower light-adapted intensity range at the 25-day time point (Fig. 5C) and amplitudes in empty-device eyes were lower than in control eyes at the two highest intensities (Fig. 5A), whereas the low-dose eyes had little if any reduction compared with fellow eyes (Fig. 5B). The pattern of reduced b-wave amplitudes in the high-dose and empty-device eyes relative to low-dose eyes (Fig. 5D) at high flash intensities is similar to the pattern at these same intensities in the dark-adapted ERG. Thus, as in the dark-adapted ERG, this suggests no consistent difference in cone b-wave amplitude between empty- and high-dose device-implanted eyes at high intensities. Statistical analysis applied to the amplitude ratios indicated a significant effect of treatment \((P = 0.05)\) and a significant interaction of treatment and intensity \((P = 0.0001)\) in the photopic ERG at 25 days (Fig. 6). Point-wise comparisons, corrected for multiple comparisons, indicated a statistically lower amplitude ratio in the high-dose group than in the empty-device group over the lowest three intensities \((P < 0.01)\), but no statistical difference between low dose and empty device at any intensity.

ONL Morphology

Light microscopic examination of the retinas revealed a substantial difference in ONL appearance in high-dose eyes compared with contralateral control eyes (Fig. 7). Morphometric measurements indicated that high-dose eyes had thicker ONLs than control fellow eyes across the entire length of the retinal sections, from the superior to the inferior ora serrata (Fig. 8C). The ONL of low-dose eyes showed a trend toward increased thickness along most of the retinal length, and empty-device eyes did not show any consistent ONL thickness trends (Figs. 8A, 8B). ONL length (Fig. 9B) was not different between groups or between treated and control eyes in the same group. The total ONL area (Fig. 9A) of the high-dose eyes was significantly greater than in control eyes (Student’s t-test, \(P < 0.05\)). Comparing across groups (Fig. 10), the one-way ANOVA indicated a significant effect of treatment on ONL area in the superior half \((P < 0.02)\) and the total retinal section \((P < 0.04)\), but not in the inferior half of the retina alone. Posttest comparison (Bonferroni’s multiple comparison test) indicated that only the high-dose eyes had a significantly greater area than the unimplanted control eyes in the superior retina \((P < 0.01)\) or the entire length of the retinal section \((P < 0.05)\). To summarize these effects, treatment with ECT-CNTF at high dose \((22 \text{ ng/eye/d})\) was associated with increased ONL thickness and area, particularly in the superior retina, but no change in overall length of the ONL.

These findings led us to examine the number of cells and cell morphology at high power in the superior retina for possible causes for the ONL area differences. Individual cell nuclei were easily distinguishable from each other, separated either by unstained area or membrane. As expected for these peripheral regions of the retina, nearly all of the cell nuclei in control ONL had the appearance of rods with densely stained chromatin in one or two large irregular clumps and little or no visible nucleolus or nuclear membrane (Fig. 7D, black arrows). By contrast the ONL nuclei in CNTF-treated retinas were more round or oval and overall lighter in appearance with the chromatin more dispersed (Fig. 7C, dark arrows). The lightly stained area surrounding the chromatin and the nuclear membrane were more prominent than in untreated eyes. In general, the cell nuclei were more conelike in appearance, and there was a reduction in the amount of space between the

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\text{Light-adapted ERG}
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\text{High dose, day 25}
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\begin{array}{c|c|c}
\text{Intensity} & \text{Control eye} & \text{Implanted} \\
\text{log cd-s/m}^2 & & \\
-1.0 & -1.0 & -1.0 \\
-0.5 & -0.5 & -0.5 \\
0 & 0 & 0 \\
1.0 & 1.0 & 1.0 \\
2.0 & 2.0 & 2.0 \\
\end{array}
\]

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\text{Background light: 34 cd/m}^2
\]
nuclei in the CNTF eye. A probable cone nucleus is indicated by the white arrows in Figures 7C and 7D. Note the large difference in size and density of chromatin.

Measurements were made in two regions approximately 135 μm in length and 100 and 1500 μm from the ora serrata. No significant difference in number of cells was found between control and device-implanted eyes in any of the groups (Student’s t-test, Table 1), though the average count was higher in high-dose eyes. However, the average photoreceptor cell nuclear area increased from 19 to 25 μm² (P < 0.0001) in high-dose eyes, an increase of approximately 25%, and from 20.5 to 21.3 μm² (P < 0.001) in low-dose eyes (Table 1). Measurements of average gray scale density (0–255 scale) in these digital images were used to quantify the differences in staining intensity. The measurements were very consistent among control retinas across groups (Table 1) and, due to the large numbers of cells measured (100–250) per group, differences of approximately 5% reached the level of significance. All device-treated eyes were significantly different from control eyes (Student’s t-test, P < 0.0001), but low-dose and high-dose CNTF-treated eyes were lower and empty-device eyes higher in staining intensity. These results indicate that ECT-CNTF delivery was associated with an increase in cross-sectional area and a decrease in average staining intensity in the photoreceptor cell nuclei in these sections.

**DISCUSSION**

This study showed no effect on rod or cone function of low-dose CNTF (5 ng/d), even though slight morphologic nuclear...
changes were observed. A higher CNTF dose (22 ng/d) caused greater changes in nuclear morphology, but caused no reduction in the rod ERG. Our data do not indicate that these changes represent a toxic effect, because functional ERG measures of the rod system over a broad range of intensities were unaffected at either dose.

Nuclear morphology changes indicate potent bioactivity of CNTF, even at the lower dose. This is in keeping with reports of CNTF promoting the survival of magnocellular neurons in the rat superoptic nucleus at 10 ng/mL and rat motorneurons at 1 to 2 ng/mL. In the study by Bok et al. an effect of dose on nuclear phenotype was inferred by comparison of the results for different vectors, promoters, and routes of administration. The controlled release of CNTF from ECT devices allowed us to relate ONL morphologic changes directly to dose (Table 1, Fig. 7) and to demonstrate that these changes have a lower dose threshold than the ERG changes. Although paraffin-embedded tissue is not ideal for the study of cell morphology, the changes in photoreceptor nuclei were apparent (Fig. 7), varied consistently with dose and the cell size differences were of the same magnitude as the differences in overall ONL area. In turn, the changes in ONL thickness and area were consistent with an effect of dose, not only across groups, but with given animals, as seen by the fact that the superior retina nearer the site of the implant, but not the inferior retina, showed a significant increase. A gradient effect of CNTF on ONL cell morphology, based on distance from the site of injection, has been reported previously. The effects of CNTF on nuclear chromatin we observed may signal the uncoiling of DNA as part of the process of gene expression.

The cone b-wave was reduced at the high dose at dim flash intensities. This effect on the photopic b-wave should be qualified further, since it originates postsynaptically to the cone photoreceptors and may contain contributions from two types of bipolar cells and Müller cells. CNTF is known to act on multiple intracellular signaling pathways in Müller cells and interneurons, but not in photoreceptors. Trophic factors, including BDNF and others, are known to be bioactive on proximal retinal cells.

The CNTF doses that we used in this study afforded protection to photoreceptors in the rd1 canine model of inherited retinal dystrophy. Major protection was achieved at an ECT dose of 5 to 15 ng/d over the course of 7 weeks, whereas 0.2 to 1 ng/d gave only minimal protection. However, one cannot make an exact comparison between the two studies. The dog eye averages 21 mm in diameter, and is larger than the rabbit eye (18 mm), giving a volume ratio of approximately 1.6. The duration of the implants was much different (25 days vs. 7 weeks). The rabbits were albino, and ocular pigmentation may affect responses to drugs. The rabbit retina is avascular, unlike the dog’s. Perhaps most important, the unhealthy photoreceptors in rd1 dogs may respond differently to CNTF than do healthy rods and cones in the rabbit.

Some of the rabbits showed vitreous membrane and engorgement of iris vessels. We subsequently found that this was due to suboptimal wound closure after surgery. Altering the suture technique resolved this problem, and we no longer capture the device titanium loop in the sclera. Employing the technique used for Vitrasert completely alleviated the problem.

CNTF given to these rabbits by ECT for 25 days did not decrease rod function at either 5 or 22 ng/d. In fact, at the lowest stimulus intensities, both the low- and high-dose eyes had higher dark-adapted b-wave amplitudes than did fellow...
control eyes (Fig. 3). In other animals, the activity of cells in the inner retina contributes substantially to the ERG at very low intensities.33,34 These ERG potentials are generated by $K^+$ currents flowing through Müller cells.35 CNTF activates multiple intracellular signaling pathways in these cells, but not in photoreceptors in mouse retina,23 and thus it is likely that CNTF affects these threshold potentials by its action on either inner retinal neurons or Müller cells. At the highest intensities, both the empty-device and the high-dose scotopic b-wave followed a similar pattern and tended to have slightly smaller amplitudes than their respective control eyes or the low-dose group (Fig. 2). In the rodent ERG the plateau in the dark-adapted b-wave V-log I function corresponds to saturation of the underlying PI mechanism, and the subsequent rise in amplitude above this intensity is due, in large part, to contributions from the cone system.36 Consequently, the smaller amplitude at high flash intensities is probably due to a difference in cone pathway responses. However, the cause of the secondary b-wave amplitude increase has not been demonstrated in the rabbit. In humans37 and in cats38 it has been attributed to other causes. Another factor to consider is the light-blocking effect of the device itself. Although this would result in a lower ratio between the two eyes, it would affect all groups equally and thus would not change the statistical results.

Several studies have shown that, although CNTF rescues photoreceptors in retinal degeneration eyes, it produces possible toxic side effects, as indicated by partially suppressed ERG responses and changes in photoreceptor nuclear morphology.10–13 Other studies of degenerating retinas have shown an increase in ERG function5 (Peterson WM, et al. IOVS 1998;39:ARVO Abstract 5149). In all of these, CNTF was administered by either viral-mediated gene transfer or single bolus injections. The substantial differences between these studies and our mode of CNTF delivery, concentration and the characteristics of the protein delivered make it difficult to compare them directly. However, as in our study, there is an indication that therapeutic dose is lower than that producing...

**Fig. 8.** Width of ONL along the vertical meridian of the eye measured at 500-μm intervals in the right (control) and left (implanted) eyes of rabbits in the empty-device (A), low-dose (B), and high-dose (C) groups. For comparison between experimental groups, all device-implanted eyes were plotted on the same graph in (D). Each point is the measurement from one to five rabbits. Error bars omitted for clarity.
these possibly toxic effects. Bok et al.\textsuperscript{11} demonstrated photoreceptor rescue in the retina at some distance from the injection site in rAAV-CMV-sDH-CNTF transfection, where CNTF concentrations would be expected to be lower. These areas did not show alteration of nuclear phenotype. Data from a study by Peterson et al. (Peterson WM, et al. \textit{IOVS} 1998;39: ARVO Abstract 5149) using an AAV-vectored CNTF construct with a lower affinity for the CNTF receptor and reexamined by Bok et al.,\textsuperscript{11} indicated clear retinal rescue without nuclear morphology changes in rhodopsin P23H mutant transgenic rats. The work of Cayouette et al.,\textsuperscript{5} which used the less efficient adenovirus vector, showed both rescue and an increase in ERG in \textit{nds} mice. The corneal ERG, which integrates responses from the entire retina, is less likely to reveal these regional effects.

We found a significant reduction in the cone ERG at dimmer flash intensities in high-dose CNTF eyes. If this is not simply an effect on ERG generation at the Müller cell or bipolar cell level as suggested earlier, it may be that cone function is more sensitive than rods to CNTF. Others have shown that CNTF can decrease both the scotopic and photopic ERG.\textsuperscript{11} However, the overexpression and abnormal intracellular distribution of CNTF in the study by Bok et al.\textsuperscript{11} using AAV viral-transferred CNTF DNA make it difficult to interpret this result. If, as suggested by our study, cones are more sensitive to functional suppression, but less sensitive to the therapeutic effects of CNTF, as suggested by others,\textsuperscript{4,5} it indicates that the mechanisms of ERG inhibition and cell rescue are separate. However, we do not know whether the site of functional suppression is on the cones or at a postphotoreceptor site, since the photopic ERG b-wave is generated by bipolar cells.

It is difficult to compare the in vivo activity of CNTF in the retinal rescue studies using single injections and viral gene transfer to the doses given by ECT or the biological activity of CNTF in other systems, because the levels were unknown in the previous retinal studies. The dose of CNTF given by intraocular injection to rescue photoreceptors in light damaged rat\textsuperscript{39} was 0.5 \(\mu\)g, or nearly 100 times the daily dose given by ECT in the rabbits. A similar dose given locally by single injection (1 \(\mu\)g) enhanced neurogenesis in mouse forebrain and supported the survival (50 ng/100 \(\mu\)g)\textsuperscript{40} or regrowth (0.1–1 \(\mu\)g)\textsuperscript{41} of axotomized retinal ganglion cells. However, because of the short half-life of CNTF in vivo (2.9 minutes in plasma of rats)\textsuperscript{42} and the obviously long-term action of CNTF, it is impossible to compare the pharmacokinetics in the two different routes of administration. In vitro studies in which the continuous-exposure dose is regulated, offer the best comparison. CNTF inhibited the differentiation of photoreceptor-like cells in rat pineal at 100 ng/mL,\textsuperscript{43} promoted the survival of magnocellular neurons in the rat suproptic nucleus at 10 ng/mL\textsuperscript{17} and rat motoneurons at 1 to 2 ng/mL.\textsuperscript{18} Of course, the active dose also depends on the type of activity, the concentration and location of receptors, and whether the action is direct or indirect. Indirect action of CNTF on photoreceptors is supported by the report by Wahlin et al.,\textsuperscript{23} showing activation of
intracellular signal pathways in interneurons and Müller cells but not photoreceptors. However, recent studies have also shown the presence of the CNTFα receptor on the outer segments of canine and rat photoreceptors, suggesting at least the possibility of a direct effect on these cells at lower dose than if the action were indirect.

We do not yet know whether either the decrement of ERG amplitude or the morphology will reverse when CNTF is withdrawn. The effect of still longer-term exposure to low, therapeutic doses of CNTF is also an important question. Most ERG studies, in which a decrease in rod function was found, were evaluated at 1 to 6 months of CNTF delivery (Matthew LaVail, personal communication, May 2002), and we cannot rule out the possibility that rabbits would suffer decreased rod function with a longer-term exposure to CNTF. However, in a recent study in rat (Timmers AM, et al. IOVS 2002;43:ARVO Abstract 2732), the rod ERG decreased within 1 to 2 weeks after CNTF delivery (i.e., within the time frame of our rabbit study). In our study, the ERG did not show a tendency toward progressive decrease of rod function between 5 and 25 days. The long-term effect of delivering CNTF to the retina using an ECT device is currently being investigated, since therapy for retinal degenerations will likely be effective only if extended over many months or years.

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References


